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L7 ANSWER 1 OF 2 CAPLUS COPYRIGHT 2008 ACS on STN
2004:60658 Document No. 140:105288 **Process** for producing a virus-inactivated thrombin preparation. Connolly, Caroline; Hardway, Christopher; Evans, David; Feldman, Peter (National Blood Authority, UK). PCT Int. Appl. WO 2004007707 A1 20040122, 40 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DE, DK, DM, DZ, EC, EE, ES, FI, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2003-GB2942 20030707. PRIORITY: GB 2002-16002 20020710.

AB A method for the preparation of virus-inactivated thrombin comprising solvent-detergent virus inactivation of a solution comprising prothrombin and factor X, loading the virus inactivated prothrombin and factor X onto an anion exchange medium, washing the medium to remove the reagents used for the solvent-detergent virus inactivation, and activating the prothrombin on the medium to form thrombin by the addition of **metal ions**, preferably calcium ions. The thrombin is then preferably selectively eluted from the anion exchange medium. Specifically, methods for activating and **purification** of prothrombin through anion exchange chromatog. using trometamol buffers, citrate-phosphate buffers, DEAE Sepharose CL6B column, Fractogel EMD-DEAE 650(S) column with the addition of calcium or magnesium ions are provided. Furthermore, the activity of **purified** thrombin, and its formulation, freeze drying and heat-treatment are also described.

L7 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2008 ACS on STN
2004:60540 Document No. 140:99534 **Processes** for the preparation of **fibrinogen**. Kingsland, Sarah; Clemmitt, Robert; Evans, David; Feldman, Peter (National Blood Authority, UK). PCT Int. Appl. WO 2004007533 A1 20040122, 41 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, CZ, DE, DE, DK, DK, DM, DZ, EC, EE, ES, FI, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2003-GB2928 20030707. PRIORITY: GB 2002-16001 20020710.

AB The use of immobilized **metal ion affinity chromatog.** for the separation of **fibrinogen** from plasminogen, for the **purification** of **fibrinogen** and at least one other protein, for example plasminogen, and for the co-**purification** of **fibrinogen** and factor XIII is disclosed.

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L11 ANSWER 1 OF 4 CAPLUS COPYRIGHT 2008 ACS on STN
2004:681323 Document No. 141:186902 **Purification** of human acid
 α -glucosidase for use in enzyme replacement therapy. Reuser, Arnold
J.; Van Der Ploeg, Ans T. (Neth.). U.S. Pat. Appl. Publ. US 20040161837
A1 20040819, 59 pp., Cont.-in-part of U.S. Ser. No. 46,180. (English).
CODEN: USXXCO. APPLICATION: US 2004-777644 20040213. PRIORITY: US
1995-1796P 19950802; US 1996-700760 19960729; US 2001-770253 20010129; US
2001-886477 20010622; US 2002-46180 20020116.

AB The invention provides methods of **purifying** lysosomal proteins, pharmaceutical compns. for use in enzyme replacement therapy, and methods of treating Pompe's disease using **purified** human acid α -glucosidase. The invention provides a method of **purifying** human acid α -glucosidase comprising: (a) applying a sample containing human acid α -glucosidase and contaminating proteins to an anion exchange or an affinity column under conditions in which the α -glucosidase binds to the column; (b) collecting an eluate enriched in α -glucosidase from the anion exchange or affinity column; (c) applying the eluate to (i) a hydrophobic interaction column under conditions in which α -glucosidase binds to the column and then collecting a further eluate further enriched in α -glucosidase, or (ii) contacting the eluate with hydroxylapatite under conditions in which α -glucosidase does not bind to hydroxylapatite and then collecting the unbound fraction enriched in α -glucosidase. Human acid α -glucosidase was **purified** from milk of transgenic mice. Clin. trials and pharmaceutical formulations containing human acid α -glucosidase for treatment of human acid α -glucosidase deficiency are described.

L11 ANSWER 2 OF 4 CAPLUS COPYRIGHT 2008 ACS on STN
2002:450373 Document No. 137:17132 **Purification** of human acid
 α -glucosidase for use in enzyme replacement therapy. Reuser, Arnold
J.; Van Der Ploeg, Ans T. (Neth.). U.S. Pat. Appl. Publ. US 20020073438
A1 20020613, 58 pp., Cont.-in-part of U.S. Ser. No. 770,253.
(English). CODEN: USXXCO. APPLICATION: US 2001-886477 20010622.
PRIORITY: US 1995-1796P 19950802; US 1998-111291P 19981207; US 2001-770253
20010129.

AB The invention provides methods of **purifying** lysosomal proteins, pharmaceutical compns. for use in enzyme replacement therapy, and methods of treating Pompe's disease using **purified** human acid α -glucosidase. The invention provides a method of **purifying** human acid α -glucosidase comprising: (a) applying a sample containing human acid α -glucosidase and contaminating proteins to an anion exchange or an affinity column under conditions in which the α -glucosidase binds to the column; (b) collecting an eluate enriched in α -glucosidase from the anion exchange or affinity column; (c) applying the eluate to (i) a hydrophobic interaction column under conditions in which α -glucosidase binds to the column and then collecting a further eluate further enriched in α -glucosidase, or (ii) contacting the eluate with hydroxylapatite under conditions in which α -glucosidase does not bind to hydroxylapatite and then collecting the unbound fraction enriched in α -glucosidase.

L11 ANSWER 3 OF 4 CAPLUS COPYRIGHT 2008 ACS on STN
1999:659489 Document No. 131:268984 Chromatographic **purification**

of human acid α -glucosidase and its use for treatment of Pompe's disease. Van Corven, Emile; Weggeman, Miranda (Pharming Intellectual Property B.V., Neth.). PCT Int. Appl. WO 9951724 A1 **19991014**, 83 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-EP2475 19990406. PRIORITY: GB 1998-7464 19980407.

AB The invention provides methods of **purifying** human acid α -glucosidase, particularly from the milk of transgenic animals. The methods employ two chromatog. steps. The first step is usually anion exchange chromatog. and the second step is hydrophobic interaction chromatog. The **purification** procedure readily generates human α -glucosidase in at least 99 % weight/weight purity. Also provided are pharmaceutical compns. and methods for using **purified** human acid α -glucosidase in treatment of patients with Pompe's disease.

L11 ANSWER 4 OF 4 CAPLUS COPYRIGHT 2008 ACS on STN
1991:488789 Document No. 115:88789 Original Reference No. 115:15179a, 15182a

Process for **purifying** a metal-binding protein using an immobilized metal **affinity chromatography** resin.

Staples, Mark A.; Pargellis, Christopher A. (Biogen, Inc., USA). PCT Int. Appl. WO 9012803 A1 **19901101**, 36 pp. DESIGNATED STATES: W: AU, CA, FI, JP, KR, NO, US; RW: AT, BE, CH, DE, DK, ES, FR, GB, IT, LU, NL, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1990-US1991 19900412. PRIORITY: US 1989-338991 19890414.

AB Metal-binding proteins are **purified** from contaminants of similar net charge and mol. weight by contacting a solution containing the protein with an immobilized metal **affinity chromatog.** resin in a buffer containing a low concentration of a weak ligand for the chelant of the resin.

The adsorbed protein is then eluted using a buffer having a high concentration of the same weak ligand, e.g. Tris. Agarose-iminodiacetic acid resins having Cu²⁺ are preferred. Chelating Sepharose 6B treated with CuCl₂ was used in the **purification** of recombinant soluble T4 (CD4) antigen from contaminating fragment Bb of complement factor B.

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L13 13272 L12 AND FIBRINOGEN

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L14 1018 L13 AND AFFINITY CHROMATOG?

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L16 38 L14 AND ZINC

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L17 0 L16 AND ZINC COLUMN

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L18 8 L16 AND PLASMINOGEN

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PROCESSING COMPLETED FOR L18
L19 5 DUP REMOVE L18 (3 DUPLICATES REMOVED)

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L19 ANSWER 1 OF 5 CAPLUS COPYRIGHT 2008 ACS on STN
2006:480814 Document No. 146:77425 Approaches to the study of N-linked glycoproteins in human plasma using lectin **affinity chromatography** and nano-HPLC coupled to electrospray linear ion trap-Fourier transform mass spectrometry. Wang, Yonghui; Wu, Shiaw-lin; Hancock, William S. (Barnett Institute, Northeastern University, Boston, MA, 02115, USA). Glycobiology, 16(6), 514-523 (English) 2006. CODEN: GLYCE3. ISSN: 0959-6658. Publisher: Oxford University Press.

AB In this publication, the authors will describe the combination of lectin **affinity chromatog.** with nano HPLC coupled to a linear ion trap Fourier transform mass spectrometer (capillary LC-LTQ/FTMS) to characterize N-linked glycosylation structures in human plasma proteins. The authors used a well-characterized glycoprotein, tissue **plasminogen** activator (rt-PA), which is present at low levels in blood, as a standard to determine the dynamic range of this approach. N-linked glycopeptides derived from rt-PA could be characterized at a ratio of 1:200 in human plasma (rtPA: Total plasma protein, weight/weight) by accurate mass measurement in the FTMS and fragmentation (MSn) in the linear ion trap. The authors demonstrated that this platform has the potential to characterize the general N-linked glycosylation structures of abundant glycoproteins present in human plasma without the requirement for antibody-based **purification**, or addnl. carbohydrate anal. protocols. This conclusion was supported by the determination of carbohydrate structures for three glycoproteins, IgG, haptoglobin, and alpha-1-acid glycoprotein, at their natural levels in a human plasma sample, but only after the lectin enrichment step.

L19 ANSWER 2 OF 5 CAPLUS COPYRIGHT 2008 ACS on STN
2002:907166 Document No. 138:322 Plasma glucosylceramide deficiency as risk factor for thrombosis and modulator of anticoagulant protein C. Griffin, John H.; Deguchi, Hiroshi; Fernandez, Jose (USA). U.S. Pat. Appl. Publ. US 20020177563 A1 20021128, 32 pp. (English). CODEN: USXXCO.
APPLICATION: US 2002-86943 20020228. PRIORITY: US 2001-272103P 20010228; US 2001-278045P 20010322.

AB The present invention has determined that exogenously added glucosylceramide (GlcCer) and other neutral glycolipids such as the homologous Glc-containing globotriaosylceramide (Gb3Cer), dose-dependently prolonged clotting times of normal plasma in the presence but not absence of APC:protein S, indicating GlcCer or Gb3Cer can enhance protein C pathway anticoagulant activity. In studies using **purified** proteins, inactivation of factor Va by APC:protein S was enhanced by GlcCer alone and by GlcCer, globotriaosylceramide, lactosylceramide, and galactosylceramide in multicomponent vesicles containing phosphatidylserine and phosphatidylcholine. Thus, the present invention provides neutral glycolipids such as GlcCer and Gb3Cer, as anticoagulant cofactors that contribute to the antithrombotic activity of the protein C pathway. The present invention has also determined that a deficiency of plasma GlcCer is a risk factor for thrombosis. Methods are provided to determine individuals at risk for thrombosis, methods of treatment as well as methods of screening for antithrombotic factors from neutral glycolipids.

1991378546. PubMed ID: 1898066. **Purification** and characterization of a fibrinolytic enzyme from venom of the southern copperhead snake (*Agkistrodon contortrix contortrix*). Guan A L; Retzios A D; Henderson G N; Markland F S Jr. (Department of Biochemistry, University of Southern California School of Medicine, Los Angeles 90033.) Archives of biochemistry and biophysics, (1991 Sep) Vol. 289, No. 2, pp. 197-207. Journal code: 0372430. ISSN: 0003-9861. Pub. country: United States. Language: English.

AB A fibrinolytic enzyme present in *Agkistrodon contortrix contortrix* (southern copperhead) venom has been **purified** by combination of CM-cellulose chromatography, molecular sieve chromatography on Sephadex G-100, p-aminobenzamidine-agarose **affinity chromatography**, and DEAE-cellulose chromatography. The enzyme, fibrolase, has a molecular weight of 23,000-24,000 and an isoelectric point of pH 6.8. It is composed of approximately 200 amino acids, possesses a blocked NH₂-terminus and contains little or no carbohydrate. The enzyme shows no activity against a series of chromogenic p-nitroanilide substrates and is not inhibited by diisopropylfluorophosphate, soybean trypsin inhibitor, Trasylol, or p-chloromercuribenzoate. However, the enzyme is a metalloproteinase since it is inhibited by EDTA, o-phenanthroline and tetraethylpentamine (a specific **zinc** chelator). Metal analysis revealed 1 mol of **zinc**/mol of protein. Study of cleavage site preference of the fibrinolytic enzyme using the oxidized B chain of insulin revealed that specificity is similar to other snake venom metalloproteinases with cleavage primarily directed to an X-Leu bond. Interestingly, unlike some other venom fibrinolytic metalloproteinases, fibrolase exhibits little if any hemorrhagic activity. The enzyme exhibits direct fibrinolytic activity and does not activate **plasminogen**. In vitro studies revealed that fibrolase dissolves clots made either from **purified fibrinogen** or from whole blood.

L19 ANSWER 4 OF 5 CAPLUS COPYRIGHT 2008 ACS on STN
1991:202466 Document No. 114:202466 Original Reference No. 114:34041a, 34044a Thrombolytic salivary **plasminogen** activators from the vampire bat *Desmodus rotundus*. Baldus, Berthold; Donner, Peter; Schleuning, Wolf Dieter; Alagon, Alejandro; Boidol, Werner; Kraetzschmar, Joern Reiner; Haendler, Bernhard Jacques; Langer, Gernot (Schering A.-G., Germany). Eur. Pat. Appl. EP 383417 A1 19900822, 49 pp. DESIGNATED STATES: R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL. (English). CODEN: EPXXDW. APPLICATION: EP 1990-250043 19900213. PRIORITY: DE 1989-3904580 19890213; DE 1989-39179492 19890217.

AB Novel **plasminogen** activators (vPA α 1, vPA α 2, vPA β , vPA γ) for use as fibrinolytics are isolated and characterized from the saliva of the vampire bat *Desmodus rotundus* and cDNAs encoding the proteins cloned. The proteins were **purified** from saliva by a combination of Zn⁺⁺ chelate **affinity chromatog.**, gel filtration, and hydroxyapatite chromatog. Binding to immobilized *Erythrina latissima* trypsin inhibitor, heparin-Sepharose, and immobilized fibrin were demonstrated. In micro-clot lysis assays the novel **plasminogen** activators were more active than tissue **plasminogen** activator, and in vitro **plasminogen** activation was also more efficient.

L19 ANSWER 5 OF 5 MEDLINE on STN
1989255263. PubMed ID: 2566603. Interaction of histidine-rich glycoprotein with human T lymphocytes. Saigo K; Shatsky M; Levitt L J; Leung L K. (Department of Medicine, Stanford University Medical School, California 94305.) The Journal of biological chemistry, (1989 May 15) Vol. 264, No. 14, pp. 8249-53. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Histidine-rich glycoprotein (HRGP), a human plasma and platelet protein,

interacts with multiple ligands in vitro, including heparin, **plasminogen**, thrombospondin, and **fibrinogen/fibrin**. In this study, the binding of HRGP to human T lymphocytes was characterized. The binding was specific, concentration-dependent, saturable, and reversible. Scatchard plot analysis revealed two classes of binding sites: the high affinity class had an apparent dissociation constant (K_d) of $1.92 \times 10(-8)$ M, with $0.92 \times 10(4)$ sites/cell, and the low affinity class had a K_d of $4.97 \times 10(-7)$ M, with $3.7 \times 10(4)$ sites/cell. HRGP binding to T cells in the presence of HRGP-depleted serum was comparable to that observed in buffer. Dot-blot analysis showed that HRGP bound to specific T cell proteins. Using both HRGP **affinity chromatography** and immunoprecipitation with affinity-purified anti-HRGP IgG, a major 56-kDa HRGP-binding protein in surface labeled T cell lysates was demonstrated. The 56-kDa protein was shown not to be related to the CD2 molecule on T cells. The binding characteristics of HRGP to T lymphocytes indicate a specific ligand-receptor interaction. This is the first demonstration of HRGP binding to a cell surface, and its binding to human T cells may play an important role in T lymphocyte biology.

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L22 ANSWER 1 OF 6      MEDLINE on STN
2008522474.    PubMed ID: 18670766. Identification of free phosphopeptides in different biological fluids by a mass spectrometry approach. Cirulli Claudia; Chiappetta Giovanni; Marino Gennaro; Mauri Pierluigi; Amoresano Angela. (Department of Organic Chemistry and Biochemistry, Federico II University of Naples, 80126, Naples, Italy. ) Analytical and bioanalytical chemistry, (2008 Sep) Vol. 392, No. 1-2, pp. 147-59. Electronic Publication: 2008-08-01. Journal code: 101134327. E-ISSN: 1618-2650. Pub. country: Germany: Germany, Federal Republic of. Language: English.
AB Human body fluids have been rediscovered in the post-genomic era as a great source of biological markers and perhaps as source of potential biomarkers of disease. Recently, it has been found that not only proteins but also peptides and their modifications can be indicators of early pathogenic processes. This paper reports the identification of free phosphopeptides in human fluids using an improved IMAC strategy coupled to iterative mass spectrometry-based scanning techniques (neutral loss, precursor ion, multiple reaction monitoring). Many peptides were detected in the enriched extract samples when submitted to the MS-integrated strategy, whereas they were not detected in the initial extract samples. The combination of the IMAC-modified protocol with selective "precursor ion" and constant "neutral loss" triple quadrupole scan modes confers a high sensitivity on the analysis, allowing rapid phosphopeptide identification and characterization, even at low concentrations. To the best of our knowledge this work represents the first report exclusively focused on the detection of free phosphorylated peptides in biological fluids.
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L22 ANSWER 2 OF 6 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on STN

2007:504137 The Genuine Article (R) Number: 156XO. A novel ATR-FTIR approach for characterisation and identification of ex situ immobilised species. Andersson, Per Ola (Reprint); Lundquist, Margaretha; Teqler, Lotta; Borjejren, Susanne; Baltzer, Lars; Osterlund, Lars. FOI NBC Def, Dept Environm & Protect, S-90182 Umea, Sweden (Reprint); Univ Uppsala, Dept Organ Chem, S-75121 Uppsala, Sweden. perola.andersson@foi.se; lars.osterlund@foi.se. CHEMPHYSCHM (2 APR 2007) Vol. 8, No. 5, pp. 712-722. ISSN: 1439-4235. Publisher: WILEY-V C H VERLAG GMBH, PO BOX 10 11 61, D-69451 WEINHEIM, GERMANY. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB We demonstrate a novel method to analyse ex situ prepared protein chips by attenuated total reflection Fourier IR spectroscopy (ATR-FTIR), which circumvents tedious functionalisation steps of internal reflection elements (IREs), and simultaneously allows for complementary measurements by other analytical techniques. This concept is proven by utilising immobilised metal affinity capture (**IMAC** (TM)) chips containing about 10 μm thick films of copolymers coated with nitrilotriacetic acid (NTA) groups, which originally was manufactured for surface enhanced laser desorption ionisation (SELDI) spectrometry. Three immobilisation steps were analysed by ATR-FTIR spectroscopy: 1) NTA complexation with nickel(II) ions 2) binding of two histidine (His)-tagged synthetic peptides of 25 (25-His6) and 48 (48-His6) amino acids to the NTA-groups and 3) attachment of a ligand, mesyl amide, to the surface-bound 48-His6. Despite interference from H_2O , both amide I and II were well resolved. Utilising peptide adsorption in the thick copolymer matrix yields a high saturation peptide concentration of approximate to 100 mg mL^{-1} and a dissociation constant of, 116 \pm 11 μm , as determined by a detailed analysis of the Langmuir adsorption isotherm. The mesyl amide ligand was directly seen in the raw ATR-FTIR spectrum with specific peaks in the fingerprint region at 7172 and 1350 cm^{-1} . Several aspects of the fine structure of the amide I bond of the peptide were analysed: influences from secondary structure, amino side chains and competing contamination product. We believe that this approach has great potential as a stand-alone or complementary analytical tool for determination of the chemical composition of functionalised surfaces. We emphasise further that with this approach no chemical treatment of IREs is needed; the chips can be regenerated and reused, and applied in other experimental set-ups.

L22 ANSWER 3 OF 6 BIOSIS COPYRIGHT (c) 2008 The Thomson Corporation on STN
2006:137267 Document No.: PREV200600133280. Serum protein profiling distinguishes BCR-ABL plus CML from normal and neutrophilia cases. Mohamedali, Azim M. [Reprint Author]; Sahu, Satyaji; Thomas, Nicholas Shaun B.; Mufti, Ghulam J.. Kings Coll London, Dept Haematol Med, London WC2R 2LS, UK. Blood, (NOV 16 2005) Vol. 106, No. 11, Part 2, pp. 298B. Meeting Info.: 47th Annual Meeting of the American-Society-of-Hematology. Atlanta, GA, USA. December 10 -13, 2005. Amer Soc Hematol. CODEN: BLOOAW. ISSN: 0006-4971. Language: English.

AB We sought to identify additional biomarkers for chronic myelogenous leukaemia (CML) that could be an aid to early diagnosis and also yield novel antigens for immunotherapy. To this end, we screened patient serum samples at presentation against hematologically normal controls as well as patients with neutrophilia using Surface Enhanced Laser Desorption/Ionization technology (SELDI; Ciphergen ProteinChip series 4000). A total of 84 retrospective and prospective serum samples were analysed: presentation - 28, reactive neutrophilia ($> 15 \times 10^9$ neutrophils/L) - 24 and hematopoietic normal controls - 33. Patients were initially screened by routine cytogenetics and in some cases with qPCR for the BCR-ABL breakpoint. The sera samples were evaluated on 4 different array surfaces and the Immobilised Metal Affinity (**IMAC**) array was chosen as it bound serum proteins that distinguished CML from normal controls. As little as 1 μl serum was sufficient for each analysis. Biomarker artefacts due to variations in sample collection procedures were

ruled out by analysing sera (n=4) from each group at the time of collection and 3 and 6 hours post collection. There were no significant differences in any of the biomarkers at any of the time points. The spectrum of proteins obtained from each of the 84 serum samples was averaged from duplicate runs per experiment. Using the Ciphergen Express program, a panel of 5 proteins were significantly differentially expressed in CML versus the reactive neutrophilia and normal hematopoietic controls ($p < 0.001$). These proteins were identified by a combination of purification techniques using Q HyperD F columns, desalting using reverse phase C-18 beads and isolating the biomarker by 1D-SDS PAGE. The biomarkers were identified by peptide mass fingerprinting and confirmed by Tandem MS sequencing. These were 1) Albumin fragment - 2.8Kd ($p < 3.5 \times 10(-5)$, ROC=0.78), 2) **Fibrinogen** fragments 5.3Kd ($p < 6.25 \times 10(-10)$, ROC=0.07) and 5.9Kd ($p < 9.6 \times 10(-8)$, ROC=0.14), 3) Complement 3a precursor fragment - 8.9Kd ($p < 0.0015$, ROC= 0.70), 4) Platelet basic protein precursor 10.2Kd ($p < 1.5 \times 10$, ROC=0.73) and 5) Lysozyme - 14.6Kd ($p=0$. ROC=0.92). Biomarkers 3, 4 and 5 were also verified by antibody capture experiments using NP-20 arrays. In a blinded test set of sera, CIVIL. normal and neutrophilia samples were correctly classified 27/28 (96%), 32/32 (100%), 20/24 (83%) respectively using a combination of the 5.3Kd, 10.2 Kd and the 14.6 Kd markers (Biomaker Pattern software). The algorithm correctly classified 21 new samples as CML (7/8) and control (10/13). The 1/8 CML was misclassified for technical reasons. Therefore, a small number of serum biomarkers in as little as 1 μ l serum can be used to distinguish between patients with CML and neutrophilia or hematopoietic normal controls. Similar analyses may be applicable to other more heterogeneous hematological malignancies.

L22 ANSWER 4 OF 6 MEDLINE on STN DUPLICATE 1
2004437894. PubMed ID: 15342217. Generation and characterization of a novel single-chain antibody fragment specific against human fibrin clots from phage display antibody library. Yan Jun Peng; Ko Ju Ho; Qi Yi Peng. (Key Laboratory of Virology, Ministry of Education, College of Life Science, Wuhan University, Hubei, PR China 430072.) Thrombosis research, (2004) Vol. 114, No. 3, pp. 205-11. Journal code: 0326377. ISSN: 0049-3848. Pub. country: United States. Language: English.

AB A novel single-chain fragment variable (scFv) antibody was developed directed against human fibrin clots by using the human single fold scFv libraries I+J (Tomlinson I+J). Three positively binding scFvs were evaluated by scFv-phage enzyme-linked immunosorbent assay (ELISA) and DNA sequencing. Then the positive scFv was expressed in soluble form in Escherichia coli HB2151 and purified by immobilized metal affinity chromatography (**IMAC**) with a yield of about 1 mg/l, the expression of soluble scFv was verified by Western blot analysis. The purified scFv could specifically recognize human fibrin clots and indicate no binding ability with human **fibrinogen** shown by ELISA. Furthermore, we will amplify the gene of the positive scFv by polymerase chain reaction (PCR) for future study of its role in diagnosis and therapy of thrombus-correlated diseases.

L22 ANSWER 5 OF 6 CAPLUS COPYRIGHT 2008 ACS on STN
2004:727694 Document No. 142:128157 Fusion expression and purification of gloschedobin, a thrombin-like enzyme from Gloydius shedaoensis in E. coli. Li, Min; Yang, Qing; Bao, Yongming; Lei, Xuyu; Xu, Jianqiang; An, Lijia (Department of Biochemical Engineering, Dalian University of Technology, Dalian, 116024, Peop. Rep. China). Wuxi Qinggong Daxue Xuebao, 22(2), 22-25 (Chinese) 2003. CODEN: WQDXF3. ISSN: 1009-038X. Publisher: Wuxi Qinggong Daxue Xuebao Bianjibu.

AB The gene of gloschedobin, a thrombin-like enzyme, from snake venom of Gloydius shedaoensis was cloned into expression vector pET32-a(+). The enzyme fused with thioredoxin at N-terminal part was expressed in E. coli BL21 (DE3) under the control of T7 lac promoter at 30°C with 1

mmol/L IPTG for 6 h. Recombinant proteins distributed in soluble and insol. cellular fractions were purified by immobilized metal affinity chromatog. (**IMAC**) resp. SDS-PAGE was used to detect the purity of the protein. Dot-blot assay and **fibrinogen**-clotting anal. showed that the purified protein is bioactive.

L22 ANSWER 6 OF 6 MEDLINE on STN DUPLICATE 2
2000145903. PubMed ID: 10679673. Adsorption of human IgG on Cu(2+)-immobilized cellulose affinity membrane: preliminary study. Hari P R; Paul W; Sharma C P. (Division of Biosurface Technology, Biomedical Technology Wing, Sree Chitra Tirunal Institute for Medical Sciences & Technology, Thiruvananthapuram 695 012, India.) Journal of biomedical materials research, (2000 May) Vol. 50, No. 2, pp. 110-3. Journal code: 0112726. ISSN: 0021-9304. Pub. country: United States. Language: English.
AB Immobilized metal ion affinity chromatography (**IMAC**) is widely used. Transition metal ions have a high affinity to some peptide sequences. We have studied the selective adsorption of human IgG from a mixture of albumin, gamma-globulin, **fibrinogen**, and IgG onto Cu(2+) ion-immobilized cellulose membrane. Although Cu(2+) ligand is selective to IgG, in general gamma-globulins also are adsorbed. The simplicity and lower cost of Cu(2+) ion-immobilized cellulose membranes may be useful for removing IgG from blood.
Copyright 2000 John Wiley & Sons, Inc.

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L23 12 L20 AND PLASMINOGEN

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L24 11 DUP REMOVE L23 (1 DUPLICATE REMOVED)

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L24 ANSWER 1 OF 11 BIOSIS COPYRIGHT (c) 2008 The Thomson Corporation on STN 2008:127505 Document No.: PREV200800141205. Expression of r-PA and Kringle2 in E. coli and the interactions with beta 2GPI. Sun, Shi -Jing; Zhang, Chun -E; Cai, Guo-Ping [Reprint Author]. Tsing Hua Univ, Grad Sch, Life Sci and Ocean Biol Lab, Shenzhen 518055, Peoples R China.
caigp@sz.tsinghua.edu.cn. Zhou, G [Editor]; Lu, Z [Editor]; Takeyama, H [Editor]; Huang, L [Editor]. (2007) pp. 350-352. Progress on Post-Genome Technologies. Publisher: PHOENIX PUBL & MEDIA NETWORK, PUBL MANSION,165 ZHONGYANG RD, NANJING, 210009, PEOPLES R CHINA.
Meeting Info.: 5th International Forum on Post-Genome Technologies. Suzhou, PEOPLES R CHINA. September 10 -11, 2007.
ISBN: 978-7-900449-37-5(S). Language: English.

AB Fragments of r-PA and Kringle2 were obtained by PCR and then recombinant plasmids, pQE30-rPA and pRSETa-K2, were constructed and transformed into E. coli strain BL21 (DE3), respectively. The expression of the target proteins was induced by 1 mmol/L IPTG and was identified by SDS-PAGE. The result showed that vast majority of the proteins was expressed in the format of inclusion body. Kringle2 and r-PA proteins were purified by immobilized metal ion-affinity chromatography (**IMAC**) from the dissolved inclusion bodies. The fibrinolytic activities of r-PA and Kringle2 were evaluated in vitro by fibrin agarose plate assay. We found that the catalytic activity of r-PA was enhanced when the concentration of beta 2GPI increased, whereas Kringle2 not. beta 2GPI was found in enzyme-linked inummosorbent assay (ELISA) to bind specially with r-PA and Kringle2, respectively.

L24 ANSWER 2 OF 11 CAPLUS COPYRIGHT 2008 ACS on STN
2006:1173099 Document No. 145:487169 Identification of proteins showing

changes in abundance or phosphorylation using stable isotope labeling in cancer diagnosis. Pope, Robert M.; Liang, Xiquan; Hajivandi, Mahbod; Leite, John (Invitrogen Corporation, USA). PCT Int. Appl. WO 2006119435 A2 20061109, 122pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IS, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2006-US17162 20060504. PRIORITY: US 2005-678119P 20050504; US 2005-678392P 20050506; US 2005-687355P 20050603.

AB Methods for identifying proteins that are differentially expressed in disease state and normal cells using stable isotope labeling are described for diagnostic use. Stable isotope labeling of cells in culture allows for the identification of a multiplicity of proteins whose differential abundance in normal and disease state cells can be indicative of the disease state. Biomarkers are identified for breast cancer, in which the biomarkers are proteins having a two-fold or greater difference in abundance between breast cancer and normal cells. Identified biomarkers can be used detection methods that can provide diagnosis, typing, staging, or prognosis of cancer, such as breast cancer, or can be used to predict the response of cancer, such as breast cancer, to one or more anti-cancer agents.

L24 ANSWER 3 OF 11 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on STN

2006:223724 The Genuine Article (R) Number: 0160W. Integrated bioprocess for the production and isolation of urokinase from animal cell culture using supermacroporous cryogel matrices. Kumar A (Reprint); Bansal V; Nandakumar K S; Galaev I Y; Roychoudhury P K R; Holmdahl R; Mattiasson B. Lund Univ, Dept Biotechnol, Ctr Chem & Chem Engn, POB 124, SE-22100 Lund, Sweden (Reprint); Lund Univ, Dept Biotechnol, Ctr Chem & Chem Engn, SE-22100 Lund, Sweden; Protista Biotechnol AB, IDEON, SE-22370 Lund, Sweden; Indian Inst Technol, Dept Biol Sci & Bioengn, Kanpur 208016, Uttar Pradesh, India; Indian Inst Technol, Dept Biochem Engn & Biotechnol, New Delhi 110016, India; Lund Univ, Biomed Ctr, Sect Med Inflamm Res, SE-22100 Lund, Sweden. Ashok.Kumar@biotek.lu.se. BIOTECHNOLOGY AND BIOENGINEERING (5 MAR 2006) Vol. 93, No. 4, pp. 636-646. ISSN: 0006-3592. Publisher: JOHN WILEY & SONS INC, 111 RIVER ST, HOBOKEN, NJ 07030 USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB An integrated cell cultivation and protein product separation process was developed using a new type of supermacroporous polyacrylamide gel, called cryogel (pAAm-cryogel) support matrix. Human fibrosarcoma HT1080 and human colon cancer HCT116 cell lines were used to secrete urokinase (an enzyme of immense therapeutic utility) into the culture medium. The secreted protein was isolated from the circulating medium using a chromatographic capture column. A pAAm cryogel support with covalently coupled gelatin (gelatin-pAAm cryogel) was used for the cultivation of anchorage dependent cells in the continuous cell culture mode in 5% carbon dioxide atmosphere. The cells were attached to the matrix within 4-6 h of inoculation and grew as a tissue sheet inside the cryogel matrix. Continuous urokinase secretion into the circulating medium was monitored as a parameter of growth and viability of cells inside the bioreactor. No morphological changes were observed in the cells eluted from the gelatin-cryogel support and re-cultured in T-flask. The gelatin-pAAm cryogel bioreactor was further connected to a pAAm cryogel column carrying Cu(II)-iminodiacetic acid (Cu(II)-IDA)-ligands (Cu(II)-IDA-pAAm cryogel), which had been optimized for the capture of urokinase from the conditioned

medium of the cell lines. Thus an automated system was built, which integrated the features of a hollow fiber reactor with a chromatographic protein separation system. The urokinase was continuously captured by the Cu(II)-IDA-pAAm cryogel column and periodically recovered through elution cycles. The urokinase activity increased from 250 PU/mg in the culture fluid to 2,310 PU/mg after recovery from the capture column which gave about ninefold purification of the enzyme. Increased productivity was achieved by operating integrated bioreactor system continuously for 32 days under product inhibition free conditions during which no back-pressure or culture contamination was observed. A total 152,600 Plough units of urokinase activity was recovered from 500 mL culture medium using 38 capture columns over a period of 32 days. (c) 2006 Wiley Periodicals, Inc.

L24 ANSWER 4 OF 11 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on STN

2006:819120 The Genuine Article (R) Number: 075JY. Recovery of urokinase from integrated mammalian cell culture cryogel bioreactor and purification of the enzyme using p-aminobenzamidine affinity chromatography. Bansal V; Roychoudhury P K; Mattiasson B; Kumar A (Reprint). Indian Inst Technol, Dept Biol Sci & Bioengn, Kanpur 208016, Uttar Pradesh, India (Reprint); Indian Inst Technol, Dept Biochem Engn & Biotechnol, New Delhi 110016, India; Lund Univ, Dept Biotechnol, Ctr Chem & Chem Engn, SE-22100 Lund, Sweden; Protista Biotechnol AB, IDEON, SE-22370 Lund, Sweden. ashokkum@iit k.ac.in. JOURNAL OF MOLECULAR RECOGNITION (JUL-AUG 2006) Vol. 19, No. 4, pp. 332-339. ISSN: 0952-3499. Publisher: JOHN WILEY & SONS LTD, THE ATRIUM, SOUTHERN GATE, CHICHESTER PO19 8SQ, W SUSSEX, ENGLAND. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB An integrated product recovery system was developed to separate urokinase from the cell culture broth of human kidney cells HT1080. Supermacroporous monolithic cryogels provided ideal matrices with respect to surface and flow properties for use as cell culture scaffold as well as for affinity chromatographic capture step of the enzyme in the integrated system. The urokinase was produced continuously in the reactor running for 4 weeks with continuous circulation of 500 ml of culture medium. The enzyme activity in the culture medium reached to 280 Plough units (PU)/mg protein. Cu(II)-iminodiacetic acid (IDA)-polyacrylamide (pAAm) cryogel column was used to capture urokinase by integrating with the gelatin-coupled pAAm-cryogel bioreactor for HT1080 cell culture. After removing the urokinase capture column from the integrated system the bound protein was eluted. The metal affinity capture step gave 4.5-fold purification of the enzyme thus achieving a specific activity of 1300 PU/mg protein. The enzyme eluate from Cu(II)-IDA-pAAm cryogel capture column was further purified on benzamidine-Sepharose affinity column. This step finally led to a homogeneous preparation of different forms of urokinase in two different elution peaks with a best urokinase activity of 13 550 PU/mg of protein. As compared to initial activity in the cell culture broth, about 26.2- and 48.4-fold increase in specific activity was achieved with enzyme yields corresponding to 32% and 35% in two different peak fractions, respectively. Native electrophoresis and SDS-PAGE showed multiple protein bands corresponding to different forms of the urokinase, which were confirmed by Western blotting and zymography. Copyright (c) 2006 John Wiley & Sons, Ltd.

L24 ANSWER 5 OF 11 MEDLINE on STN DUPLICATE 1
2006079551. PubMed ID: 16368104. Supermacroporous cryogel matrix for integrated protein isolation. Immobilized metal affinity chromatographic purification of urokinase from cell culture broth of a human kidney cell line. Kumar Ashok; Bansal Vibha; Andersson Jonatan; Roychoudhury Pradip K; Mattiasson Bo. (Department of Biotechnology, Center for Chemistry and Chemical Engineering, Lund University, P.O. Box 124, SE-22100 Lund,

Sweden.) Journal of chromatography. A, (2006 Jan 20) Vol. 1103, No. 1, pp. 35-42. Electronic Publication: 2005-12-20. Journal code: 9318488. ISSN: 0021-9673. Pub. country: Netherlands. Language: English.

AB A new type of supermacroporous, monolithic, cryogel affinity adsorbent was developed, allowing the specific capture of urokinase from conditioned media of human fibrosarcoma cell line HT1080. The affinity adsorbent was designed with the objective of using it as a capture column in an integrated perfusion/protein separation bioreactor setup. A comparative study between the utility of this novel cryogel based matrix and the conventional Sepharose based affinity matrix for the continuous capture of urokinase in an integrated bioreactor system was performed. Cu(II)-ion was coupled to epoxy activated polyacrylamide cryogel and Sepharose using iminodiacetic acid (IDA) as the chelating ligand. About 27-fold purification of urokinase from the conditioned culture media was achieved with Cu(II)-IDA-polyacrylamide cryogel column giving specific activity of about 814 Plough units (PU)/mg protein and enzyme yields of about 80%. High yields (95%) were obtained with Cu(II)-IDA-Sepharose column by virtue of its high binding capacity. However, the adsorbent showed lower selectivity as compared to cryogel matrix giving specific activity of 161 PU/mg protein and purification factor of 5.3. The high porosity, selectivity and reasonably good binding capacity of Cu(II)-IDA-polyacrylamide cryogel column make it a promising option for use as a protein capture column in integrated perfusion/separation processes. The urokinase peak pool from Cu(II)-IDA-polyacrylamide cryogel column could be further resolved into separate fractions for high and low molecular weight forms of urokinase by gel filtration chromatography on Sephadex S-200. The selectivity of the cryogel based **IMAC** matrix for urokinase was found to be higher as compared to that of Cu(II)-IDA-Sepharose column.

L24 ANSWER 6 OF 11 CAPLUS COPYRIGHT 2008 ACS on STN

2004:204049 Document No. 140:249735 Cell culture, lysis and chromatog. purifn. methods for production of adenovirus vectors carrying cloned therapeutic genes. Senesac, Joseph (Introgen Therapeutics Inc., USA). PCT Int. Appl. WO 2004020971 A2 20040311, 250 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2003-US26831 20030827.

PRIORITY: US 2002-406591P 20020828.

AB This invention provides methods for purification of clin. grade adenovirus from cell lysate by two-column chromatog. in addition to other purification steps. Also disclosed are methods for the high-yield production of adenovirus vectors by large-scale cell culture or bioreactor. Methods and materials for cell lysis and recovery of adenoviruses are disclosed. Adenovirus vectors carrying cloned therapeutic transgenes may be produced and purified by the methods of the invention.

L24 ANSWER 7 OF 11 CAPLUS COPYRIGHT 2008 ACS on STN

2004:558500 Document No. 141:294715 Transfected insect cells in suspension culture rapidly yield moderate quantities of recombinant proteins in protein-free culture medium. Farrell, Patrick; Iatrou, Kostas (Pharmaceutical Production Research Facility, Faculty of Engineering, University of Calgary, Calgary, AB, T2N 1N4, Can.). Protein Expression and Purification, 36(2), 177-185 (English) 2004. CODEN: PEXPEJ. ISSN: 1046-5928. Publisher: Elsevier Science.

AB Methodol. to rapidly express milligram quantities of recombinant proteins through the Lipofectin-mediated transfection of insect cells in

small-scale, protein-free suspension culture is presented. The transfection phase in suspension culture was first optimized using the green fluorescence protein coupled with FACs anal. to examine the effect of variables such as the transfection media, duration, and cell d. on transfection efficiency and expression level. The recombinant protein production phase was optimized using secreted alkaline phosphatase (SEAP) as a reporter protein to evaluate the cell seeding d. and harvest time. Using this method, 5 secreted, 2 intracellular, and 1 chimeric protein were expressed at levels ranging from 6 to 50 mg/L. Furthermore, the ability to purify over 2 mg of His6-tagged SEAP by immobilized metal affinity chromatog. from 50 mL insect cell culture medium to greater than 95% purity was also demonstrated. This method is suitable for scale-up and high-throughput applications.

L24 ANSWER 8 OF 11 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on STN

2004:680257 The Genuine Article (R) Number: 839TK. Chromatographic purification of an insoluble histidine tag recombinant Ykt6p SNARE from *Arabidopsis thaliana* over-expressed in E-coli. Vincent P; Dieryck W; Maneta-Peyret L; Moreau P; Cassagne C; Santarelli X (Reprint). Univ Bordeaux 2, ESTBB, 146 Rue Leo Saignat, F-33076 Bordeaux, France (Reprint); Univ Bordeaux 2, ESTBB, F-33076 Bordeaux, France; Univ Bordeaux 2, CNRS, Lab Biogenese Membranaire, UMR 5544, F-33076 Bordeaux, France. xavier.santarelli@estbb.u-bordeaux2.fr. JOURNAL OF CHROMATOGRAPHY B-ANALYTICAL TECHNOLOGIES IN THE BIOMEDICAL AND LIFE SCIENCES (25 AUG 2004) Vol. 808, No. 1, Sp. iss. SI, pp. 83-89. ISSN: 1570-0232. Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB In order to undertake in plant cell the study of the endoplasmic reticulum (ER)-Golgi apparatus (GA) protein and/or lipid vesicular transport pathway, expressed sequence tag (EST) coding for a homologue to the yeast soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs) Ykt6p has been cloned in *Arabidopsis thaliana* by reverse transcription polymerase chain reaction (RT-PCR). The corresponding protein was over-expressed as a recombinant histidine-tag (his-tag) protein in *E. coli*. Starting from one litter of culture, an ultrasonic homogenization was performed for cell disruption and after centrifugation the *Arabidopsis* Ykt6p SNARE present in inclusion bodies in the pellet was solubilized. After centrifugation, the clarified feedstock obtained was injected onto an immobilized metal affinity chromatography (**IMAC**) in presence of 6 M guanidine and on-column refolding was performed. Folded and subsequently purified (94% purity) recombinant protein was obtained with 82% of recovery. (C) 2004 Elsevier B.V. All rights reserved.

L24 ANSWER 9 OF 11 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on STN

1997:381744 The Genuine Article (R) Number: WZ027. Immobilized metal-ion affinity chromatography: Imidazole proton pump and chromatographic sequelae .1. Proton pump. Sulkowski E. ROSWELL PK CANC INST, DEPT MOL & CELLULAR BIOL, BUFFALO, NY 14263. JOURNAL OF MOLECULAR RECOGNITION (SEP-DEC 1996) Vol. 9, No. 5-6, pp. 389-393. ISSN: 0952-3499. Publisher: JOHN WILEY & SONS LTD, BAFFINS LANE CHICHESTER, W SUSSEX, ENGLAND PO19 1UD. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Complexation of imidazole (Im) with an iminodiacetate (IDA) metal chelate [IDA-M(II)] ligand of a chelating gel results in an acidification of the mobile phase. The scope of the action of this IDA-M(II)Im 'proton pump' in **IMAC** is determined by: (a) IDA-M(II) density of the gel; (b) concentration of applied Im; and (c) the buffering capacity of the mobile phase. Application of Im onto a metal chelate column in a

gradient rather than in a stepwise manner, mitigates the proton pump's action, as it does an increase of buffer concentration in the mobile phase. However, only an antecedent conversion of the metal chelate gel, IDA-M(II), to its Im derivative, IDA-M(II) Im, can effectively circumscribe the action of the proton pump. The same holds true, as anticipated, when another chelating ligand (nitrilotriacetate) is used.

L24 ANSWER 10 OF 11 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on STN

1991:148267 The Genuine Article (R) Number: FA785. IMMOBILIZED METAL-ION AFFINITY PARTITIONING, A METHOD COMBINING METAL PROTEIN-INTERACTION AND PARTITIONING OF PROTEINS IN AQUEOUS 2-PHASE SYSTEMS. BIRKENMEIER G (Reprint); VIJAYALAKSHMI M A; STIGBRAND T; KOPPERSCHLAGER G. KARL MARX UNIV, INST BIOCHEM, LIEBIGSTR 16, D-7010 LEIPZIG, GERMANY (Reprint); UNIV TECHNOL COMPIEGNE, TECHNOL SEPARAT LAB, F-60206 COMPIEGNE, FRANCE; UMEA UNIV, INST PHYSIOL CHEM, S-90187 UMEA, SWEDEN. JOURNAL OF CHROMATOGRAPHY (22 FEB 1991) Vol. 539, No. 2, pp. 267-277. ISSN: 0021-9673. Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Immobilized metal ions were used for the affinity extraction of proteins in aqueous two-phase systems composed of polyethylene glycol (PEG) and dextran or PEG and salt. Soluble chelating polymers were prepared by covalent attachment of metal-chelating groups to PEG. The effect on the partitioning of proteins of such chelating PEG derivatives coordinated with different metal ions is demonstrated. The proteins studied were alpha₂-macroglobulin, tissue **plasminogen** activator, superoxide dismutase and monoclonal antibodies. The results indicate that immobilized metal ion affinity partitioning provides excellent potential for the extraction of proteins.

L24 ANSWER 11 OF 11 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on STN

1991:667259 The Genuine Article (R) Number: GT220. IMMOBILIZED METAL-ION AFFINITY-CHROMATOGRAPHY (**IMAC**) CHEMISTRY AND BIOSEPARATION APPLICATIONS. WONG J W (Reprint); ALBRIGHT R L; WANG N H L. PURDUE UNIV, SCH CHEM ENGN, W LAFAYETTE, IN 47907 (Reprint); ROHM & HAAS CO, RES LABS, SPRING HOUSE, PA 19477; UNIV CALIF DAVIS, DAVIS, CA 95618. SEPARATION AND PURIFICATION METHODS (1991) Vol. 20, No. 1, pp. 49-106. ISSN: 0360-2540. Publisher: MARCEL DEKKER INC, 270 MADISON AVE, NEW YORK, NY 10016. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB This review discusses the principles of immobilized metal ion affinity chromatography (**IMAC**) and its applications to protein separations. **IMAC** functions by binding the accessible electron-donating pendant groups of a protein - such as histidine, cysteine, and tryptophan - to a metal ion which is held by a chelating group covalently attached on a stationary support. A common chelating group is iminodiacetate. The ions commonly used are of borderline or soft metals, such as Cu²⁺, Ni²⁺, Co²⁺, and Zn²⁺. Protein retention in **IMAC** depends on the number and type of pendant groups which can interact with the metal. The interaction is affected by a variety of independent variables such as pH, temperature, solvent type, salt type, salt concentration, nature of immobilized metal and chelate, ligand density, and protein size. Proteins are usually eluted by a decreasing pH gradient or by an increasing gradient of a competitive agent, such as imidazole, in a buffer. There are still several unresolved issues in **IMAC**. The exact structures of protein-immobilized metal complexes need to be known so that retention behavior of proteins can be fully understood and sorbent structures can be optimized. Engineering parameters, such as adsorption/desorption rate constants, sorbent capacities, and intraparticle diffusivities, need to be developed for most

protein systems. Engineering analysis and quantitative understanding are also needed so that **IMAC** can be used efficiently for large scale protein separations.

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L25 0 L20 AND BLOOD FACTOR XIII

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L26 1 L20 AND FACTOR XIII

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L26 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2008 ACS on STN
2006:513356 Document No. 144:495255 Purification of recombinant human

factor XIII. Bransford, Carol; Cheema, Hardarshan; Hogg, Deborah; Meng, Wenmao; O'Donnell, Ray; Robertson, Ewan; Topping, Andrew (ZymoGenetics, Inc., USA). PCT Int. Appl. WO 2006056575 A1 20060601, 31 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IS, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2005-EP56169 20051123. PRIORITY: US 2004-630614P 20041123.

AB The present invention provides improved methods for the purification of **factor XIII.** In particular, the methods provide compns. containing 5 % or less contaminating proteins. In particular embodiments of the present invention the methods provide purified **factor XIII** compns. comprising less than 1 % activated **factor XIII**, less than 2 % protein aggregates, and/or less than 5 % charge isomers of **factor XIII.** The methods do not require the use a precipitation or crystallization step common to prior methods of isolating **factor XIII.** Instead, the method uses immobilized metal affinity chromatog. to remove various contaminants common to recombinant expression of **factor XIII.** Further, a combination of various chromatog. methods including ion exchange chromatog., hydrophobic affinity chromatog., and immobilized metal affinity chromatog. comprise a simple and less expensive method to produce a pharmaceutical grade **factor XIII** product at high yield.

=> s co-eluting
L27 1519 CO-ELUTING

=> s 127 and fibrinogen
L28 0 L27 AND FIBRINOGEN

=> s 127 and factor XIII
L29 0 L27 AND FACTOR XIII

=> s (kingsland s?/au or clemmitt r?/au or evans d?/au or feldman p?/au)
L30 30266 (KINGSLAND S?/AU OR CLEMMITT R?/AU OR EVANS D?/AU OR FELDMAN P?/AU)

=> s 130 and fibrinogen
L31 55 L30 AND FIBRINOGEN

=> s 131 and plasminogen
L32 1 L31 AND PLASMINOGEN

=> d 132 cbib abs

L32 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2008 ACS on STN
2004:60540 Document No. 140:99534 Processes for the preparation of
fibrinogen. **Kingsland, Sarah; Clemmitt, Robert**
; **Evans, David; Feldman, Peter** (National Blood
Authority, UK). PCT Int. Appl. WO 2004007533 A1 20040122, 41 pp.
DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY,
BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DE, DK, DM, DZ, EC, EE, EE,
ES, FI, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP,
KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI,
NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SK, SL, SY, TJ,
TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE,
BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT,
LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN:
PIXXD2. APPLICATION: WO 2003-GB2928 20030707. PRIORITY: GB 2002-16001
20020710.

AB The use of immobilized metal ion affinity chromatog. for the separation of
fibrinogen from **plasminogen**, for the purification of
fibrinogen and at least one other protein, for example
plasminogen, and for the co-purification of **fibrinogen** and
factor XIII is disclosed.

=> s 131 and pd<20020710
1 FILES SEARCHED...
4 FILES SEARCHED...
L33 48 L31 AND PD<20020710

=> s 133 and metal ion
L34 0 L33 AND METAL ION

=> s 133 and copper
L35 0 L33 AND COPPER

=> s 133 and zinc
L36 0 L33 AND ZINC

=> s 133 and nickel column
L37 0 L33 AND NICKEL COLUMN

=> s 133 and purif?
L38 6 L33 AND PURIF?

=> dup remove 138
PROCESSING COMPLETED FOR L38
L39 4 DUP REMOVE L38 (2 DUPLICATES REMOVED)

=> d 139 1-4 cbib abs

L39 ANSWER 1 OF 4 EMBASE COPYRIGHT (c) 2008 Elsevier B.V. All rights
reserved on STN
1990094857 EMBASE A small-scale model of factor VIII and factor IX
fractionation from plasma.
Feldman, P.; Winkelman, L.; Evans, H.; Pinnell, M.; Murdoch, F.;
Smith, J.K.. Plasma Fractionation Laboratory, Churchill Hospital, Oxford
OX3 7LJ, United Kingdom.
Transfusion Science Vol. 10, No. 4, pp. 279-286 1989.

ISSN: 0955-3886. CODEN: TRASEE.

Pub. Country: United Kingdom. Language: English. Summary Language: English.

Entered STN: 911213. Last Updated on STN: 911213

AB A small-scale model of factor VIII and factor IX fractionation from human plasma has been developed. Validation experiments demonstrate that it accurately reflects processing at pilot scale. Tests of reproducibility show that there is a greater agreement between pools composed of plasma from the same donors than of random donors, but to predict the performance of pilot and manufacturing scale fractionation several pools from different donor panels must be tested.

L39 ANSWER 2 OF 4 MEDLINE on STN

1989389312. PubMed ID: 2506696. Severely heated therapeutic factor VIII concentrate of high specific activity. Winkelmann L; Owen N E; **Evans D R**; Evans H; Haddon M E; Smith J K; Prince P J; Williams J D; Lane R S. (Plasma Fractionation Laboratory, Churchill Hospital, Oxford, UK.) Vox sanguinis, (1989) Vol. 57, No. 2, pp. 97-103. Journal code: 0413606. ISSN: 0042-9007. Pub. country: Switzerland. Language: English.

AB A new method for the manufacture of a heated factor VIII concentrate of high specific activity (2-6 IU factor VIII:C/mg protein) has been developed. Addition of heparin to cryoprecipitate extract at acid pH precipitated **fibrinogen** and fibronectin. Factor VIII was then recovered from the supernatant by precipitation with glycine and sodium chloride. After re-solution and desalting on Sephadex G-25, the concentrate was sterile-filtered and lyophilised. The dried product was stable to heating in the final container at 80 degrees C for 72 h. Data from 25 consecutive batches of concentrate prepared from 1,200-1,500 kg plasma pools are presented. The mean final yield of heated product was 190 IU factor VIII:C/kg plasma. The concentrate has been found to be safe and effective in clinical use.

L39 ANSWER 3 OF 4 MEDLINE on STN

DUPLICATE 1

1986316633. PubMed ID: 3750269. A pasteurised concentrate of human plasma factor XIII for therapeutic use. Winkelmann L; Sims G E; Haddon M E; **Evans D R**; Smith J K. Thrombosis and haemostasis, (1986 Jun 30) Vol. 55, No. 3, pp. 402-5. Journal code: 7608063. ISSN: 0340-6245. Pub. country: GERMANY, WEST: Germany, Federal Republic of. Language: English.

AB A therapeutic concentrate of factor XIII containing both A and B sub-units has been prepared from 300 kg pools of human plasma. The process starts from a cold-ethanol fraction from cryoprecipitate supernatant and therefore does not interfere with the recovery of other clinically valuable plasma proteins. Factor XIII is **purified** approximately 600-fold from plasma by precipitation with sodium citrate and by the removal of **fibrinogen** by brief heating. The product has been pasteurised in sorbitol solution to inactivate blood-borne viruses, ultrafiltered to remove sorbitol, adsorbed with bentonite and freeze-dried in a formulation meeting requirements for intravenous injection.

L39 ANSWER 4 OF 4 CAPLUS COPYRIGHT 2008 ACS on STN

1977:473289 Document No. 87:73289 Original Reference No. 87:11619a,11622a Improved factor VIII concentrate of intermediate purity. Ellis, D.; Pettet, N.; Williams, J. D.; Maycock, W. d'A.; Smith, J. K.; **Evans, D. R.**; Bidwell, E. (Blood Prod. Lab., Lister Inst. Prev. Med., Elstree/Herts., UK). British Journal of Haematology, 36(1), 149-50 (English) 1977. CODEN: BJHEAL. ISSN: 0007-1048.

AB The properties and approx. composition of an improved Factor VIII [9001-27-8] concentrate are described. The Factor VIII was obtained by 2 modified procedures which had an addnl. **purification** stage (compared to the old procedure), in which the contaminating proteins were precipitated from the Factor VIII extract by manipulating pH and temperature. No new ions or precipitants

were introduced by this procedure. The dried products thus prepared dissolved in approx. the same time as the earlier concs. and had similar compns. of ions. Sp. activity was increased but the ratio of **fibrinogen** to other proteins remained unchanged. Though the new concs. had a much higher potency (11-15 IU/mL) than the old concs. (4.0-5.5 IU/mL), they were described as being in the intermediate-purity category.

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CA SUBSCRIBER PRICE	-12.80	-12.80

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NEWS 6 APR 26 USPATFULL and USPAT2 enhanced with patent assignment/reassignment information
NEWS 7 APR 28 CAS patent authority coverage expanded
NEWS 8 APR 28 ENCOMPLIT/ENCOMPLIT2 search fields enhanced
NEWS 9 APR 28 Limits doubled for structure searching in CAS REGISTRY
NEWS 10 MAY 08 STN Express, Version 8.4, now available

NEWS 11 MAY 11 STN on the Web enhanced
NEWS 12 MAY 11 BEILSTEIN substance information now available on STN Easy
NEWS 13 MAY 14 DGENE, PCTGEN and USGENE enhanced with increased limits for exact sequence match searches and introduction of free HIT display format
NEWS 14 MAY 15 INPADOCDB and INPAFAMDB enhanced with Chinese legal status data
NEWS 15 MAY 28 CAS databases on STN enhanced with NANO super role in records back to 1992
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AND CURRENT DISCOVER FILE IS DATED 06 APRIL 2009.

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FULL ESTIMATED COST ENTRY 0.22 0.22

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=> s metal ion affinity chromatography
L1 1970 METAL ION AFFINITY CHROMATOGRAPHY

=> s 11 and plasminogen
L2 8 L1 AND PLASMINOGEN

=> s 12 and fibrinogen
L3 1 L2 AND FIBRINOGEN

=> d 13 cbib abs

L3 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2009 ACS on STN
2004:60540 Document No. 140:99534 Processes for the preparation of
fibrinogen. Kingsland, Sarah; Clemmitt, Robert; Evans, David;
Feldman, Peter (National Blood Authority, UK). PCT Int. Appl. WO
2004007533 A1 20040122, 41 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT,
AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, CZ, DE,
DE, DK, DM, DZ, EC, EE, ES, FI, FI, GB, GD, GE, GH, GM, HR, HU,
ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA,
MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC,
SD, SE, SG, SK, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC,
VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK,
ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD,
TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2003-GB2928 20030707.
PRIORITY: GB 2002-16001 20020710.

AB The use of immobilized **metal ion affinity chromatog**. for the separation of **fibrinogen** from
plasminogen, for the purification of **fibrinogen** and at least
one other protein, for example **plasminogen**, and for the
co-purification of **fibrinogen** and factor XIII is disclosed.

=> s 12 and pd<20030707

1 FILES SEARCHED...

4 FILES SEARCHED...

L4 6 L2 AND PD<20030707

=> dup remove 14

PROCESSING COMPLETED FOR L4

L5 3 DUP REMOVE L4 (3 DUPLICATES REMOVED)

=> d 15 1-3 cbib abs

L5 ANSWER 1 OF 3 EMBASE COPYRIGHT (c) 2009 Elsevier B.V. All rights
reserved on STN DUPLICATE 1
1997056088 EMBASE High-level secretion in *Pichia pastoris* and biochemical
characterization of the recombinant kringle 2 domain of tissue-type
plasminogen activator.
Nilsen, Stephanie L.; DeFord, Melanie E.; Prorok, Mary; Chibber, Bakshy A.
K.; Bretthauer, Roger K.; Castellino, Francis J. (correspondence).
Department of Chemistry and Biochemistry, Center for Transgene Research,
University of Notre Dame, Notre Dame, IN 46556, United States. Castellino,
Francis J. (correspondence). Dept. of Chemistry and Biochemistry,
University of Notre Dame, Notre Dame, IN 46556, United States.
Biotechnology and Applied Biochemistry Vol. 25, No. 1, pp. 63-74
1997.

Refs: 23.

ISSN: 0885-4513. CODEN: BABIEC.

Pub. Country: United Kingdom. Language: English. Summary Language:
English.

Entered STN: 970324. Last Updated on STN: 970324

AB The kringle 2 (K2) domain of tissue-type **plasminogen** activator
(tPA) has been expressed in *Pichia pastoris* cell lines GS115 and KM71.
This construct contained a hexahistidine sequence at the C-terminus of the
kringle to aid in purification by immobilized **metal-ion**
-affinity chromatography. The exact amino acid
sequence of the isolated kringle was EAEAYV[K2(tPA)]SR(H)(6), where
[K2(tPA)] represents amino acid sequence residues C(1)-C(82) of the
kringle domain (residues 180-261 of tPA). The clones of the yeast
transformants provided large amounts of the recombinant

(r)-[K2(tPA)]-containing polypeptide at levels that allowed ready purification of several hundred mg from shake flasks and near-gram levels from a high-biomass fermenter. Purification of the kringle domain directly from cell-conditioned media was accomplished in a single step by either immobilized Ni(+) affinity chromatography or lysine-Sepharose affinity chromatography. N-linked glycans were present on approx. 30% of this yeast-expressed material, at N(5) of the kringle (corresponds to N(11) of the particular construct, N(184) of full-length tPA). The expressed recombinant kringle recognized a conformation-specific monoclonal antibody generated against tPA that is directed to the K2 domain of the protein, interacted properly with various ω -amino acid ligands, and showed signature conformational properties when studied by differential scanning calorimetry and high-resolution $^1\text{H-NMR}$. The results demonstrate that the *P. pastoris* system can be employed to obtain large amounts of secreted and properly folded kringle domains.

L5 ANSWER 2 OF 3 SCISEARCH COPYRIGHT (c) 2009 The Thomson Corporation on STN

1997:381744 The Genuine Article (R) Number: WZ027. Immobilized **metal -ion affinity chromatography**: Imidazole proton pump and chromatographic sequelae .1. Proton pump. Sulkowski E. ROSWELL PK CANC INST, DEPT MOL & CELLULAR BIOL, BUFFALO, NY 14263. JOURNAL OF MOLECULAR RECOGNITION (**SEP-DEC 1996**) Vol. 9, No. 5-6, pp. 389-393. ISSN: 0952-3499. Publisher: JOHN WILEY & SONS LTD, BAFFINS LANE CHICHESTER, W SUSSEX, ENGLAND PO19 1UD. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Complexation of imidazole (Im) with an iminodiacetate (IDA) metal chelate [IDA-M(II)] ligand of a chelating gel results in an acidification of the mobile phase. The scope of the action of this IDA-M(II)Im 'proton pump' in IMAC is determined by: (a) IDA-M(II) density of the gel; (b) concentration of applied Im; and (c) the buffering capacity of the mobile phase. Application of Im onto a metal chelate column in a gradient rather than in a stepwise manner, mitigates the proton pump's action, as it does an increase of buffer concentration in the mobile phase. However, only an antecedent conversion of the metal chelate gel, IDA-M(II), to its Im derivative, IDA-M(II) Im, can effectively circumscribe the action of the proton pump. The same holds true, as anticipated, when another chelating ligand (nitrilotriacetate) is used.

L5 ANSWER 3 OF 3 SCISEARCH COPYRIGHT (c) 2009 The Thomson Corporation on STN

1991:667259 The Genuine Article (R) Number: GT220. IMMobilized **METAL -ION AFFINITY-CHROMATOGRAPHY** (IMAC) CHEMISTRY AND BIOSEPARATION APPLICATIONS. WONG J W (Reprint). PURDUE UNIV, SCH CHEM ENGN, W LAFAYETTE, IN 47907 (Reprint). ALBRIGHT R L; WANG N H L. ROHM & HAAS CO, RES LABS, SPRING HOUSE, PA 19477; UNIV CALIF DAVIS, DAVIS, CA 95618. SEPARATION AND PURIFICATION METHODS (**1991**) Vol. 20, No. 1, pp. 49-106. ISSN: 0360-2540. Publisher: MARCEL DEKKER INC, 270 MADISON AVE, NEW YORK, NY 10016. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB This review discusses the principles of immobilized **metal ion affinity chromatography** (IMAC) and its applications to protein separations. IMAC functions by binding the accessible electron-donating pendant groups of a protein - such as histidine, cysteine, and tryptophan - to a metal ion which is held by a chelating group covalently attached on a stationary support. A common chelating group is iminodiacetate. The ions commonly used are of borderline or soft metals, such as Cu²⁺, Ni²⁺, Co²⁺, and Zn²⁺. Protein retention in IMAC depends on the number and type of pendant groups which can interact with the metal. The interaction is affected by a variety of

independent variables such as pH, temperature, solvent type, salt type, salt concentration, nature of immobilized metal and chelate, ligand density, and protein size. Proteins are usually eluted by a decreasing pH gradient or by an increasing gradient of a competitive agent, such as imidazole, in a buffer. There are still several unresolved issues in IMAC. The exact structures of protein-immobilized metal complexes need to be known so that retention behavior of proteins can be fully understood and sorbent structures can be optimized. Engineering parameters, such as adsorption/desorption rate constants, sorbent capacities, and intraparticle diffusivities, need to be developed for most protein systems. Engineering analysis and quantitative understanding are also needed so that IMAC can be used efficiently for large scale protein separations.

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=> s 11 and toyopearl AF chelate 650M
L6          0 L1 AND TOYOPEARL AF CHELATE 650M

=> s plasminogen
L7          198438 PLASMINOGEN

=> s 17 and fibrinogen
L8          23846 L7 AND FIBRINOGEN

=> s 18 and fractogel
L9          5 L8 AND FRACTOGEL

=> dup remove 19
PROCESSING COMPLETED FOR L9
L10         3 DUP REMOVE L9 (2 DUPLICATES REMOVED)

=> d 110 1-3 cbib abs

L10 ANSWER 1 OF 3 CAPLUS COPYRIGHT 2009 ACS on STN
2005:983807 Document No. 143:272378 Fibrinogen purification with
cation exchangers or hydrophobic or dye gels. Metzner, Hubert; Liebing,
Uwe; Feussner, Annette; Lemmer, Joerg; Schulte, Stefan; Gawantka, Volker
(Germany). U.S. Pat. Appl. Publ. US 20050197493 A1 20050908, 20 pp.
(English). CODEN: USXXXCO. APPLICATION: US 2005-62432 20050223.
PRIORITY: DE 2004-102004669400 20040224.

AB The present invention relates to a process for purifying
fibrinogen, which comprises one or more process steps in which one
or more contaminating proteins are depleted by neg. chromatog. and/or neg.
adsorption using cation exchanger, hydrophobic gel and/or dye gel. In
addition, the invention relates to the fibrinogen which is obtained
by the process of the invention and which is notable for improved
stability, and to the production and use of pharmaceutical preps. comprising
this fibrinogen.

L10 ANSWER 2 OF 3 CAPLUS COPYRIGHT 2009 ACS on STN
2005:954035 Document No. 143:253862 Purification of fibrinogen
using negative chromatography. Metzner, Hubert; Liebing, Uwe; Feussner,
Annette; Lemmer, Joerg; Schulte, Stefan; Gawantka, Volker (ZLB Behring
G.m.b.H., Germany). Eur. Pat. Appl. EP 1568709 A2 20050831, 27 pp.
DESIGNATED STATES: R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL,
SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, PL,
SK, BA, HR, IS, YU. (German). CODEN: EPXXDW. APPLICATION: EP 2005-3017
20050212. PRIORITY: DE 2004-102004009400 20040224.

AB The invention concerns a method for the purification of fibrinogen
from fibrinogen-containing solns. in several steps; at least one
step includes neg. chromatog. or neg. adsorption using cation exchangers,
hydrophobic gels or dye gels. Chromatog. is performed in the presence of
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agents that decrease the binding of **plasminogen** with **fibrinogen**. An addnl. purification step is precipitation. Blood products, milk from transgenic animals or fermentation products are purified. Fibrin adhesives

and matrixes are produced; the formulations contain sodium chloride, sodium citrate, arginine, arginine hydrochloride or calcium chloride; fibrin products have good storage stability.

L10 ANSWER 3 OF 3 MEDLINE on STN DUPLICATE 1

1990385460. PubMed ID: 2402747. Isolation of human **fibrinogen** and its derivatives by affinity chromatography on Gly-Pro-Arg-Pro-Lys-**Fractogel**. Kuyas C; Haeberli A; Walder P; Straub P W. (Department of Medicine, University of Bern, Inselspital, Switzerland.) Thrombosis and haemostasis, (1990 Jun 28) Vol. 63, No. 3, pp. 439-44. Journal code: 7608063. ISSN: 0340-6245. Pub. country: GERMANY, WEST: Germany, Federal Republic of. Language: English.

AB With an immobilized synthetic pentapeptide GlyProArgProLys comprising the N-terminal sequence GlyProArg of the alpha-chain of fibrin, a new affinity method for the quantitative isolation of **fibrinogen** out of anticoagulated plasma was developed. The method proved to be superior to all known isolation methods in respect to ease of use and yield, since **fibrinogen** could be isolated in one step out of plasma with a recovery of more than 95% when compared to the immunologically measurable amounts of **fibrinogen**. Moreover the amounts of contaminating proteins such as fibronectin, factor XIII or **plasminogen** were negligible and the purity of the isolated **fibrinogen** was higher than 95% as measured by polyacrylamide gel electrophoresis. The clottability was 90% and more. Another advantage of this affinity purification method is the possibility to isolate **fibrinogen** quantitatively out of small plasma samples (less than 5 ml). Further, abnormal **fibrinogen** molecules, provided their complementary binding site for GlyProArg is preserved, may also be quantitatively isolated independent of any solubility differences as compared to normal **fibrinogen**. In addition fibrin(ogen) fragments originating from plasmic digestion can be separated on the basis of their affinity to GlyProArg. The described affinity gel can be used more than 50 times without any loss of capacity.

=> s 18 and toyopearl AF chelate 650M column

L11 0 L8 AND TOYOPEARL AF CHELATE 650M COLUMN

=> s 18 and Toyopearl

L12 2 L8 AND TOYOPEARL

=> dup remove l12

PROCESSING COMPLETED FOR L12

L13 2 DUP REMOVE L12 (0 DUPLICATES REMOVED)

=> d 113 1-2 cbib abs

L13 ANSWER 1 OF 2 CAPLUS COPYRIGHT 2009 ACS on STN

2006:193617 Document No. 144:270173 Sequential schemes for isolation and purification of plasma proteins by affinity chromatography for pharmaceutical use. Burton, Steven James; Baines, Baldev; Curling, John; Hayes, Timothy Keith; Chen, Dwun-Hou; Bryant, Christopher; Hammond, David John (Prometic Biosciences Ltd., UK; American National Red Cross). PCT Int. Appl. WO 2006023831 A2 20060302, 40 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT,

RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IS, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2005-US29739 20050819. PRIORITY: US 2004-602868P 20040820.

AB The invention discloses methods for sequential plasma protein isolation (e.g., recombinant plasma proteins from cell culture) and purification from a biol. sample by affinity chromatog. for pharmaceutical use. The target protein comprises **fibrinogen**, α -1 proteinase inhibitor, apolipoprotein A1, IgG, paraoxonase, coagulation factors, von Willebrand factor, serum albumin, and **plasminogen**. Affinity chromatog. is conducted using ligands or ligand support complexes that selectively and specifically bind to proteins in the biol. sample. The ligands or ligand support complexes were contacted sequentially in a predetd. order with the biol. sample to allow each ligand or ligand-support complex to sequentially bind a protein from the biol. sample. Various linear cascade sequence scenarios were defined and plasma buffering systems were evaluated and selected for use in linear cascade processes.

L13 ANSWER 2 OF 2 MEDLINE on STN

2001519482. PubMed ID: 11566257. The cell envelope-bound metalloprotease (camelysin) from *Bacillus cereus* is a possible pathogenic factor. Fricke B; Drossler K; Willhardt I; Schierhorn A; Menge S; Rucknagel P. (Institute of Physiological Chemistry, Medical Faculty, Martin Luther University, Halle, Saale, Germany.. beate.fricke@medizin.uni-halle.de) . Biochimica et biophysica acta, (2001 Sep 28) Vol. 1537, No. 2, pp. 132-46. Journal code: 0217513. ISSN: 0006-3002. Pub. country: Netherlands. Language: English.

AB A novel membrane proteinase of the nosocomial important bacteria species *Bacillus cereus* (synonyms: camelysin, CCMP) was purified up to homogeneity as was shown by mass spectrometry in its amphiphilic form. Camelysin is a neutral metalloprotease with a molecular mass of 19 kDa. Its unique N-terminus Phe-Phe-Ser-Asp-Lys-Glu-Val-Ser-Asn-Asn-Thr-Phe-Ala-Ala-Gly-Thr-Leu-Asp-Leu-Thr-Leu-Asn-Pro-Lys-Thr-Leu-Val-Asp-(Ile-Lys-Asp)- was not detected in the protein data bases during BLAST searches, but in the partially sequenced genome of *Bacillus anthracis*, coding for an unknown protein. Cleavage sites of the membrane proteinase for the insulin A- and B-chains were determined by mass spectrometry and N-terminal sequencing. Camelysin prefers cleavage sites in front of aliphatic and hydrophilic amino acid residues (-OH, -SO₃H, amido group), avoiding bulky aromatic residues. The internally quenched fluorogenic substrates of the matrix metalloproteases 2 and 7 were cleaved with the highest efficiency at the Leu-decrease-Gly or Leu-decrease-Ala bond with the smaller residue in the P1' position. The protein specificity is broad--all various kinds of casein were cleaved as well as acid-soluble collagen, globin and ovalbumin; intact insulin was destroyed only to a low extent. Actin, collagen type I, **fibrinogen**, fibrin, alpha2-antiplasmin and alpha1-antitrypsin were cleaved. The protease formed SDS-stable complexes with Glu-**plasminogen** and antithrombin III, visible after SDS-electrophoresis by gold staining and Western blot. The CCMP-**plasminogen** complex caused a partial activation of **plasminogen** to plasmin. Camelysin interacts with proteins of the blood coagulation cascade and could facilitate the penetration of fibrin clots and of the extracellular matrix during bacterial invasion.

=> s 18 and copper

L14 147 L8 AND COPPER

=> s 114 and chromatography

L15 12 L14 AND CHROMATOGRAPHY

=> dup remove l15
PROCESSING COMPLETED FOR L15
L16 9 DUP REMOVE L15 (3 DUPLICATES REMOVED)

=> d 116 1-9 cbib abs

L16 ANSWER 1 OF 9 CAPLUS COPYRIGHT 2009 ACS on STN
2006:885955 Document No. 145:290486 Protein markers for the diagnosis, prognosis, monitoring, and selection of therapy of CNS lymphoma.
Rubenstein, James; Schulman, Howard; Becker, Christopher H.; Roy, Sushmita Mimi (Ppd Biomarker Discovery Sciences, LLC, USA). PCT Int. Appl. WO 2006091861 A2 20060831, 740 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IS, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2006-US6681 20060224. PRIORITY: US 2005-656749P 20050225.

AB Polypeptide markers are provided that are identified as differentially expressed in central nervous system (CNS) lymphoma samples, including cerebrospinal fluid samples from patients with CNS lymphoma, as compared to CSF samples obtained from control patients without cancer. The markers are also differentially expressed in patients with carcinomatous meningitis and metastatic brain cancers. Many of the polypeptides are fragments of complete proteins, either because they were present as fragments in the sample or as a result of the trypsin digestion that was performed during the processing of certain fractions of the sample. The tryptic peptides prepared from a high-mol.-weight fraction of cerebrospinal fluid were profiled by liquid chromatog.-electrospray ionization-mass spectrometry on a high-resolution time-of-flight (TOF) instrument. Polypeptide markers with particular statistical significance are identified as antithrombin III, complement factor H, or epidermal growth factor-containing fibulin-like extracellular matrix protein 1 (EFEMP1, also known as fibulin-3).

L16 ANSWER 2 OF 9 CAPLUS COPYRIGHT 2009 ACS on STN
2004:681323 Document No. 141:186902 Purification of human acid α -glucosidase for use in enzyme replacement therapy. Reuser, Arnold J.; Van Der Ploeg, Ans T. (Neth.). U.S. Pat. Appl. Publ. US 20040161837 A1 20040819, 59 pp., Cont.-in-part of U.S. Ser. No. 46,180. (English). CODEN: USXXCO. APPLICATION: US 2004-777644 20040213. PRIORITY: US 1995-1796P 19950802; US 1996-700760 19960729; US 2001-770253 20010129; US 2001-886477 20010622; US 2002-46180 20020116.

AB The invention provides methods of purifying lysosomal proteins, pharmaceutical compns. for use in enzyme replacement therapy, and methods of treating Pompe's disease using purified human acid α -glucosidase. The invention provides a method of purifying human acid α -glucosidase comprising: (a) applying a sample containing human acid α -glucosidase and contaminating proteins to an anion exchange or an affinity column under conditions in which the α -glucosidase binds to the column; (b) collecting an eluate enriched in α -glucosidase from the anion exchange or affinity column; (c) applying the eluate to (i) a hydrophobic interaction column under conditions in which α -glucosidase binds to the column and then collecting a further eluate further enriched in α -glucosidase, or (ii) contacting the eluate with hydroxylapatite under conditions in which α -glucosidase does not bind to hydroxylapatite and then collecting the unbound fraction enriched in α -glucosidase. Human acid α -glucosidase was purified from milk of transgenic mice. Clin. trials and pharmaceutical

formulations containing human acid α -glucosidase for treatment of human acid α -glucosidase deficiency are described.

L16 ANSWER 3 OF 9 CAPLUS COPYRIGHT 2009 ACS on STN
2002:907166 Document No. 138:322 Plasma glucosylceramide deficiency as risk factor for thrombosis and modulator of anticoagulant protein C. Griffin, John H.; Deguchi, Hiroshi; Fernandez, Jose (USA). U.S. Pat. Appl. Publ. US 20020177563 A1 20021128, 32 pp. (English). CODEN: USXXCO.
APPLICATION: US 2002-86943 20020228. PRIORITY: US 2001-272103P 20010228; US 2001-278045P 20010322.

AB The present invention has determined that exogenously added glucosylceramide (GlcCer) and other neutral glycolipids such as the homologous Glc-containing globotriaosylceramide (Gb3Cer), dose-dependently prolonged clotting times of normal plasma in the presence but not absence of APC:protein S, indicating GlcCer or Gb3Cer can enhance protein C pathway anticoagulant activity. In studies using purified proteins, inactivation of factor Va by APC:protein S was enhanced by GlcCer alone and by GlcCer, globotriaosylceramide, lactosylceramide, and galactosylceramide in multicomponent vesicles containing phosphatidylserine and phosphatidylcholine. Thus, the present invention provides neutral glycolipids such as GlcCer and Gb3Cer, as anticoagulant cofactors that contribute to the antithrombotic activity of the protein C pathway. The present invention has also determined that a deficiency of plasma GlcCer is a risk factor for thrombosis. Methods are provided to determine individuals at risk for thrombosis, methods of treatment as well as methods of screening for antithrombotic factors from neutral glycolipids.

L16 ANSWER 4 OF 9 CAPLUS COPYRIGHT 2009 ACS on STN
2002:450373 Document No. 137:17132 Purification of human acid α -glucosidase for use in enzyme replacement therapy. Reuser, Arnold J.; Van Der Ploeg, Ans T. (Neth.). U.S. Pat. Appl. Publ. US 20020073438 A1 20020613, 58 pp., Cont.-in-part of U.S. Ser. No. 770,253. (English). CODEN: USXXCO. APPLICATION: US 2001-886477 20010622. PRIORITY: US 1995-1796P 19950802; US 1998-111291P 19981207; US 2001-770253 20010129.

AB The invention provides methods of purifying lysosomal proteins, pharmaceutical compns. for use in enzyme replacement therapy, and methods of treating Pompe's disease using purified human acid α -glucosidase. The invention provides a method of purifying human acid α -glucosidase comprising: (a) applying a sample containing human acid α -glucosidase and contaminating proteins to an anion exchange or an affinity column under conditions in which the α -glucosidase binds to the column; (b) collecting an eluate enriched in α -glucosidase from the anion exchange or affinity column; (c) applying the eluate to (i) a hydrophobic interaction column under conditions in which α -glucosidase binds to the column and then collecting a further eluate further enriched in α -glucosidase, or (ii) contacting the eluate with hydroxylapatite under conditions in which α -glucosidase does not bind to hydroxylapatite and then collecting the unbound fraction enriched in α -glucosidase.

L16 ANSWER 5 OF 9 CAPLUS COPYRIGHT 2009 ACS on STN
2001:62437 Document No. 134:97520 Method for detecting low density lipoprotein (LDL) or denatured LDL in blood. Uchida, Kazuo; Mashiba, Shinichi (Ikagaku Co., Ltd., Japan). Eur. Pat. Appl. EP 1070962 A2 20010124, 23 pp. DESIGNATED STATES: R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO. (English). CODEN: EPXXDW. APPLICATION: EP 2000-114984 20000720. PRIORITY: JP 1999-207913 19990722; JP 2000-12210 20000120.

AB A novel method for detecting LDL and denatured LDL (particularly, oxidized LDL) having a significant concern with the onset and progression of arteriosclerosis and Alzheimer's disease is provided, wherein a complex of denatured LDL (particularly, oxidized LDL) with an acute phase reactant,

blood coagulation-fibrinolytic-related protein or disinfectant substance produced by macrophage is used as a measuring subject. Human LDL free of α_1 antitrypsin and human fibronectin were treated with a **copper** sulfate solution at 37° over night to form an oxidized LDL-fibronectin complex. The complex was used as an immunogen in a mouse from which monoclonal antibodies were prepared for use in assaying for the complex.

L16 ANSWER 6 OF 9 CAPLUS COPYRIGHT 2009 ACS on STN
2001:38027 Document No. 134:350344 Characterization and pathogenetic role of proteinase from Acanthamoeba castellanii. Na, Byoung-Kuk; Kim, Jae-Chan; Song, Chul-Yong (Department of Biology, College of Natural Sciences, Chung-Ang University, Seoul, 156-756, S. Korea). Microbial Pathogenesis, 30(1), 39-48 (English) 2001. CODEN: MIPAEV. ISSN: 0882-4010. Publisher: Academic Press.

AB A secreted proteinase was purified from the culture supernatant of Acanthamoeba castellanii with several **chromatog.** steps. The purified proteinase was a chymotrypsin-like serine proteinase. Its mol. weight was approx. 12 kDa on SDS-PAGE, and its native mol. weight was 12 kDa when determined by mol. sieve **chromatog.** It showed a broad temperature optimum ranging 30-55° with an optimal at 55° and an optimal pH of 8.5. It could degrade various protein substrates, such as collagen, fibronectin, laminin, secretory IgA, IgG, **plasminogen**, **fibrinogen**, Hb, and rabbit corneal proteins. It showed strong cytopathic effects in cultured cells, including HEp2 and HEK cells. The corneal lesions, induced by both the purified proteinase and A. castellanii, displayed similar clin. results for both cases, in which the stromal infiltration and opacity with the epithelial defect were revealed. These results suggest that the enzyme was highly associated with the pathogenesis of Acanthamoeba. The fact that cytopathic effects and development of corneal lesions caused by the proteinase of Acanthamoeba were inhibited by the proteinase inhibitor suggest that the proteinase inhibitor might be useful as a therapeutic agent. (c) 2001 Academic Press.

L16 ANSWER 7 OF 9 CAPLUS COPYRIGHT 2009 ACS on STN
1999:659489 Document No. 131:268984 Chromatographic purification of human acid α -glucosidase and its use for treatment of Pompe's disease. Van Corven, Emile; Weggeman, Miranda (Pharming Intellectual Property B.V., Neth.). PCT Int. Appl. WO 9951724 A1 19991014, 83 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2.
APPLICATION: WO 1999-EP2475 19990406. PRIORITY: GB 1998-7464 19980407.

AB The invention provides methods of purifying human acid α -glucosidase, particularly from the milk of transgenic animals. The methods employ two **chromatog.** steps. The first step is usually anion exchange **chromatog.** and the second step is hydrophobic interaction **chromatog.** The purification procedure readily generates human α -glucosidase in at least 99 % weight/weight purity. Also provided are pharmaceutical compns. and methods for using purified human acid α -glucosidase in treatment of patients with Pompe's disease.

L16 ANSWER 8 OF 9 MEDLINE on STN DUPLICATE 1
1999383905. PubMed ID: 10452958. Characterization and purification of an outer membrane metalloproteinase from Pseudomonas aeruginosa with fibrinogenolytic activity. Fricke B; Parchmann O; Kruse K; Rucknagel P;

Schierhorn A; Menge S. (Institute of Physiological Chemistry, Medical Faculty, Martin Luther University, 06097, Halle (Saale), Germany.. beate.fricke@medizin.uni-halle.de) . Biochimica et biophysica acta, (1999 Aug 30) Vol. 1454, No. 3, pp. 236-50. Journal code: 0217513. ISSN: 0006-3002. Pub. country: Netherlands. Language: English.

AB A membrane proteinase from *Pseudomonas aeruginosa*, called insulin-cleaving membrane proteinase (ICMP), was located in the outer membrane leaflet of the cell envelope. The enzyme is expressed early in the logarithmic phase parallel to the bacterial growth during growth on peptide rich media. It is located with its active center facing to the outermost side of the cell, because its whole activity could be measured in intact cells. The very labile membrane proteinase was solubilized by non-ionic detergents (Nonidet P-40, Triton X-100) and purified in its amphiphilic form to apparent homogeneity in SDS-PAGE by **copper** chelate **chromatography** and two subsequent chromatographic steps on Red-Sepharose CL-4B (yield 58.3%, purification factor 776.3). It consisted of a single polypeptide chain with a molecular mass of 44.6 kDa, determined by mass spectrometry. ICMP was characterized to be a metalloprotease with pH-optimum in the neutral range. The ICMP readily hydrolyzed Glu(13)-Ala(14) and Tyr(16)-Leu(17) bonds in the insulin B-chain. Phe(25)-Tyr(26) and His(10)-Leu(11) were secondary cleavage sites suggesting a primary specificity of the enzyme for hydrophobic or aromatic residues at P'(1)-position. The ICMP differed from elastase, alkaline protease and LasA in its cleavage specificity, inhibition behavior and was immunologically diverse from elastase. The amino acid sequence of internal peptides showed no homologies with the known proteinases. This outer membrane proteinase was capable of specific cleavage of alpha and beta **fibrinogen** chains. Among the p-nitroanilide substrates tested, substrates of **plasminogen** activator, complement convertase and kallikrein with arginine residues in the P(1)-subsite were the substrates best accepted, but they were only cleaved at a very low rate.

L16 ANSWER 9 OF 9 CAPLUS COPYRIGHT 2009 ACS on STN
1991:488789 Document No. 115:88789 Original Reference No. 115:15179a,15182a
Process for purifying a metal-binding protein using an immobilized metal affinity **chromatography** resin. Staples, Mark A.; Pargellis, Christopher A. (Biogen, Inc., USA). PCT Int. Appl. WO 9012803 A1
19901101, 36 pp. DESIGNATED STATES: W: AU, CA, FI, JP, KR, NO, US; RW:
AT, BE, CH, DE, DK, ES, FR, GB, IT, LU, NL, SE. (English). CODEN:
PIXXD2. APPLICATION: WO 1990-US1991 19900412. PRIORITY: US 1989-338991
19890414.

AB Metal-binding proteins are purified from contaminants of similar net charge and mol. weight by contacting a solution containing the protein with an immobilized metal affinity **chromatog.** resin in a buffer containing a low concentration of a weak ligand for the chelant of the resin. The adsorbed protein is then eluted using a buffer having a high concentration of the same weak ligand, e.g. Tris. Agarose-iminodiacetic acid resins having Cu²⁺ are preferred. Chelating Sepharose 6B treated with CuCl₂ was used in the purification of recombinant soluble T4 (CD4) antigen fom contaminating fragment Bb of complement factor B.

=> s 18 and arginine
L17 458 L8 AND ARGININE

=> s 17 and trisodium citrate
L18 16 L7 AND TRISODIUM CITRATE

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PROCESSING COMPLETED FOR L18

L19 11 DUP REMOVE L18 (5 DUPLICATES REMOVED)

=> d 119 1-11 cbib abs

L19 ANSWER 1 OF 11 CAPLUS COPYRIGHT 2009 ACS on STN
2007:1275603 Document No. 147:482469 Diagnosis of thromboembolic venous diseases by D-dimer and soluble fibrin determination. Mirshahi, Bibi Shah Soltan; Soria, Jeannette (Diagnostica Stago, Fr.; Assistance Publique Hopitaux de Paris). Fr. Demande FR 2900734 A1 20071109, 44pp. (French). CODEN: FRXXBL. APPLICATION: FR 2006-4072 20060505.

AB The invention concerns a method and test for the determination of coagulation activation, especially in relation with thromboembolic venous diseases; the method includes the determination of D-dimers and soluble fibrins at the time of fibrinolysis activation in a blood sample. The method is based on comparing the D-dimer rate corresponding to the soluble fibrin degradation and the D-dimer rate in the patient's sample with values from healthy individuals' blood samples. The test can also be used to monitor anticoagulation treatment.

L19 ANSWER 2 OF 11 SCISEARCH COPYRIGHT (c) 2009 The Thomson Corporation on STN

2007:767142 The Genuine Article (R) Number: 193PN. Concentration of heparin-locking solution and risk of central venous hemodialysis catheter malfunction.

Hemmelgarn, Brenda R. (Reprint). Foothills Med Ctr, Div Nephrol, 1403 29th St NW, Calgary, AB T2N 2T9, Canada (Reprint). Thomas, Chandra M.; Zhang, Jianguo; Lim, Teik How; Scott-Douglas, Nairne; Hons, Ronald B.. Corporate Author: Alberta Kidney Dis Network. Univ Calgary, Dept Med, Div Nephrol, Calgary, AB, Canada; Univ Calgary, Dept Community Hlth Sci, Calgary, AB, Canada.

ASAIO JOURNAL (JUL-AUG 2007) Vol. 53, No. 4, pp. 485-488. ISSN: 1058-2916. Publisher: LIPPINCOTT WILLIAMS & WILKINS, 530 WALNUT ST, PHILADELPHIA, PA 19106-3621 USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Heparin is used as an interdialytic locking solution for hemo-dialysis (HD) central venous catheters (CVCs). The purpose of this study was to compare effectiveness of two heparin concentrations (10,000 and 1,000 U/mL) in preventing catheter malfunction. We compared two time periods: a 6-month period with heparin 10,000 U/mL and a 3-month period with heparin 1,000 U/mL. Adults on HD using a CVC (tunneled or un tunneled) in Calgary, Alberta, were included. The primary outcome was catheter malfunction. A total of 139 and 134 patients in the heparin 10,000 and 1,000 U/mL periods, respectively, were included. The crude rate of catheter malfunction, per 1,000 HD sessions, was similar for heparin 10,000 (7.6; 95% CI, 5.3 to 10.8) and 1,000 (6.7; 95% CI, 4.3 to 10.3) U/mL periods, respectively ($p = 0.76$). After adjusting for CVC characteristics and use of recombinant tissue plasminogen activator (rt-PA), there was no association between heparin concentration and CVC malfunction (hazard ratio, 0.77; 95% CI, 0.37 to 1.61). In conclusion, the use of a lower concentration of heparin was not associated with an increased risk of catheter malfunction but may be associated with greater rt-PA use. The association between heparin concentration and rt-PA use requires further study. ASAIO Journal 2007; 53:485-488.

L19 ANSWER 3 OF 11 EMBASE COPYRIGHT (c) 2009 Elsevier B.V. All rights reserved on STN DUPLICATE 1

2007066235 EMBASE Trisodium citrate 4% - An alternative to heparin capping of haemodialysis catheters.

Lok, Charmaine E., Dr. (correspondence); Appleton, Debra; Bhola, Cynthia; Khoo, Brian; Richardson, Robert M.A.. University Health Network, Toronto General Hospital, Toronto, ON, Canada. charmaine.lok@uhn.on.ca. Lok,

Charmaine E., Dr. (correspondence); Appleton, Debra; Bhola, Cynthia; Khoo, Brian; Richardson, Robert M.A.. University of Toronto, Toronto, ON, Canada . charmaine.lok@uhn.on.ca. Lok, Charmaine E., Dr. (correspondence). Department of Medicine, Division of Nephrology, The Toronto General Hospital, 200 Elizabeth Street, Toronto, ON M5G 2C4, Canada. charmaine.lok@uhn.on.ca.

Nephrology Dialysis Transplantation Vol. 22, No. 2, pp. 477-483 Feb 2007.

Refs: 36.

ISSN: 0931-0509. E-ISSN: 1460-2385. CODEN: NDTREA.

Pub. Country: United Kingdom. Language: English. Summary Language: English.

Entered STN: 20070228. Last Updated on STN: 20070906

AB Background: Central venous catheters (CVCs) continue to be used at a high rate for dialysis access and are frequently complicated by thrombus-related malfunction. Prophylactic locking with an anticoagulant, such as heparin, has become standard practice despite its associated risks. **Trisodium citrate** (citrate) 4% is an alternative catheter locking anticoagulant. Methods: The objective was to prospectively study the clinical effectiveness, safety and cost of citrate 4% vs heparin locking by comparing rates of CVC exchanges, thrombolytic use (TPA) and access-associated hospitalizations during two study periods: heparin period (HP) (1 June 2003-15 February 2004) and Citrate Period (CP) 15 March-15 November 2004. Incident catheters evaluated did not overlap the two periods. Results: There were 176 CVC in 121 patients (HP) and 177 CVC in 129 patients (CP). The event rates in incident CVC were: CVC exchange 2.98/ 1000 days (HP) vs 1.65/1000 days (CP) (P = 0.01); TPA use 5.49/1000 (HP) vs 3.3/1000 days (CP) (P = 0.002); hospitalizations 0.59/1000 days (HP) vs 0.28/1000 days (CP) (P = 0.49). There was a longer time from catheter insertion to requiring CVC exchange (P = 0.04) and TPA (P = 0.006) in the citrate compared with the heparin lock group. Citrate locking costs less than heparin locking but a formal economic analysis including indirect costs was not done. Conclusion: Citrate 4% has equivalent or better outcomes with regards to catheter exchange, TPA use and access-related hospitalizations compared with heparin locking. It is a safe and less expensive alternative. Randomized trials comparing these anticoagulants with a control group would definitively determine the optimal haemodialysis catheter locking solution. .COPYRGT. 2007 Oxford University Press.

L19 ANSWER 4 OF 11 MEDLINE on STN DUPLICATE 2
2006445187. PubMed ID: 16627606. Catheter lock solutions influence staphylococcal biofilm formation on abiotic surfaces. Shanks Robert M Q; Sargent Jennifer L; Martinez Raquel M; Gruber Martha L; O'Toole George A. (Department of Microbiology and Immunology, Dartmouth Medical School, Hanover, NH 03755, USA.) Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association, (2006 Aug) Vol. 21, No. 8, pp. 2247-55. Electronic Publication: 2006-04-20. Journal code: 8706402. ISSN: 0931-0509. Pub. country: England: United Kingdom. Language: English.

AB BACKGROUND: Microbial biofilms form on central venous catheters and may be associated with systemic infections as well as decreased dialysis efficiency due to catheter thrombosis. The most widely used anticoagulant catheter lock solution in the US is sodium heparin. We have previously shown that sodium heparin in clinically relevant concentrations enhances *Staphylococcus aureus* biofilm formation. In the present study, we examine the effect of several alternative catheter lock solutions on *in vitro* biofilm formation by laboratory and clinical isolates of *S. aureus* and coagulase-negative staphylococci (CNS). METHODS: Lepirudin, low molecular weight heparin, tissue **plasminogen** activator, sodium citrate, sodium citrate with gentamicin and sodium ethylene diamine tetra-acetic acid (EDTA) were assessed for their effect on biofilm formation on

polystyrene, polyurethane and silicon elastomer. RESULTS: Sodium citrate at concentrations above 0.5% efficiently inhibits biofilm formation and cell growth of *S. aureus* and *Staphylococcus epidermidis*. Subinhibitory concentrations of sodium citrate significantly stimulate biofilm formation in most tested *S. aureus* strains, but not in CNS strains. Sodium EDTA was effective in prevention of biofilm formation as was a combination of sodium citrate and gentamicin. Low molecular weight heparin stimulated biofilm formation of *S. aureus*, while lepirudin and tissue **plasminogen** activator had little effect on *S. aureus* biofilm formation. CONCLUSIONS: This *in vitro* study demonstrates that heparin alternatives, sodium citrate and sodium EDTA, can prevent the formation of *S. aureus* biofilms, suggesting that they may reduce the risk of biofilm-associated complications in indwelling catheters. This finding suggests a biological mechanism for the observed improvement in catheter-related outcomes in recent clinical comparisons of heparin and **trisodium citrate** as catheter locking solutions. A novel and potential clinically relevant finding of the present study is the observation that citrate at low levels strongly stimulates biofilm formation by *S. aureus*.

L19 ANSWER 5 OF 11 EMBASE COPYRIGHT (c) 2009 Elsevier B.V. All rights reserved on STN
2006518170 EMBASE Catheter lock solutions: It's time for a change.
Mandolfo, Salvatore; Borlandelli, S.; Elli, A.. Renal Unit, Maggiore Hospital, Viale Savoia, 26900 Lodi, Italy. nefrodialisi.lodi@ao.lodi.it.
Madolfo, S., Dr. (correspondence). Renal Unit, Maggiore Hospital, Viale Savoia, 26900 Lodi, Italy. nefrodialisi.lodi@ao.lodi.it.
Journal of Vascular Access Vol. 7, No. 3, pp. 99-102 Jul 2006.
Refs: 28.

ISSN: 1129-7298. CODEN: JVAOAI.
Pub. Country: Italy. Language: English. Summary Language: English.
Entered STN: 20061113. Last Updated on STN: 20061113

AB In recent years, the number of patients on hemodialysis (HD) with central vascular catheters (CVCs) has grown. However, CVC use is often associated with an important risk for catheter related bloodstream infections (CRBI) and inadequate dialysis due to flow problems. In this study, we reviewed alternative solutions to heparin for locking HD CVCs. Several experiences have demonstrated that **trisodium citrate** (TSC) (30-47%), citrate (4%) and taurolidine (1.35%) solutions are effective and safe for the prevention of CRBI, while heparin stimulates biofilm formation. High citrate (47%) concentrations can also provide significant advantages in reducing catheter clotting, but controlled studies with larger populations are necessary to confirm and to extend the use of such solutions in clinical practice. Side effects with high sodium citrate concentrations have been reported only immediately after locking, the symptoms are probably caused by a temporary drop in ionized calcium and magnesium, but it is evident that these solutions should only be used by skilled and authorized personnel, with a rigorous protocol. .COPYRGT.
Wichtig Editore, 2006.

L19 ANSWER 6 OF 11 CAPLUS COPYRIGHT 2009 ACS on STN
2005:983807 Document No. 143:272378 Fibrinogen purification with cation exchangers or hydrophobic or dye gels. Metzner, Hubert; Liebing, Uwe; Feussner, Annette; Lemmer, Joerg; Schulte, Stefan; Gawantka, Volker (Germany). U.S. Pat. Appl. Publ. US 20050197493 A1 20050908, 20 pp. (English). CODEN: USXXXCO. APPLICATION: US 2005-62432 20050223.

PRIORITY: DE 2004-102004669400 20040224.
AB The present invention relates to a process for purifying fibrinogen, which comprises one or more process steps in which one or more contaminating proteins are depleted by neg. chromatog. and/or neg. adsorption using cation exchanger, hydrophobic gel and/or dye gel. In addition, the invention relates to the fibrinogen which is obtained by the process of the

invention and which is notable for improved stability, and to the production and use of pharmaceutical preps. comprising this fibrinogen.

L19 ANSWER 7 OF 11 CAPLUS COPYRIGHT 2009 ACS on STN

2002:752251 Document No. 137:2685080 Prosthetic devices comprising biocidal locks. Prosl, Frank R.; Estabrook, Brian K.; Sodemann, Klaus (Biolink Corporation, USA). Eur. Pat. Appl. EP 1245247 A1 20021002, 46 pp. DESIGNATED STATES: R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR. (English). CODEN: EPXXDW. APPLICATION: EP 2001-107955 20010328.

AB Disclosed herein is an internal prosthetic device comprising: (a) means for providing a continuous flow-path, crossing a patient skin, between an external-to-patient site and an internal-to-patient site; (b) means for blocking the flow-path; and (c) a biocidal lock comprising: (i) an anticoagulant; and (ii) a non-antibiotic biocide. A 0.5% solution of taurolidine in Ringer-lactate solution was introduced into each of 4 polyethylene bottles having a 30-mL volume. One bottle was filled with 5 mL of the taurolidine solution and 2 mL ACD-A solution. ACD-A solution is used for the conservation of whole blood and contains/L: 22.0 g sodium citrate dihydrate, 7.3 g citric acid and 34.5 g glucose monohydrate. Blood was collected a from a female pig directly from the slaughter wound into the containers that were then filled up to the 30-mL level. Blood in the containers containing only taurolidine was clotted, but the blood in the container containing the mixture of taurolidine and ACD-A was not clotted. Thus, the use of sodium citrate and citric acid anticoagulants in combination with taurolidine provides substantially enhanced anticoagulant properties in whole blood.

L19 ANSWER 8 OF 11 CAPLUS COPYRIGHT 2009 ACS on STN

2001:489485 Document No. 135:58156 Separation of fibrinogen from plasma proteases by extraction and ion exchange chromatography. Kanellos, Jerry; Kleinig, Michael; Martinelli, Teresa (CSL Limited, Australia). PCT Int. Appl. WO 2001048016 A1 20010705, 70 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2000-AU1585 20001221. PRIORITY: AU 1999-4842 19991223; AU 1999-4841 19991223.

AB The present invention relates to methods for purifying fibrinogen. In one aspect, the present invention relates to a method of separating fibrinogen from plasma fraction I precipitate. In another aspect, the invention relates to the purification of fibrinogen using ion exchange chromatog. The extraction conditions

recommended for fraction 1 paste are 20 mM tri-sodium citrate, 0.8 M NaCl, 5 mM ϵ -amino caproic acid, 60 IU/mL heparin, pH 7.3, extracted for 90 min at 37°.

L19 ANSWER 9 OF 11 BIOSIS COPYRIGHT (c) 2009 The Thomson Corporation on STN 2000:415720 Document No.: PREV200000415720. S31922 is a new orally active

thrombin inhibitor with low food interaction. Rupin, A. [Reprint author]; Vallez, M. O. [Reprint author]; Versluys, D. [Reprint author]; De Nanteuil, G.; Verbeuren, T. J. [Reprint author]. Division of Angiology, Institut de Recherches Servier, Suresnes, France. Haemostasis, (May, 2000) Vol. 30, No. 1-2, pp. 54. print.

Meeting Info.: 1st North Sea Conference on Thrombosis and Haemostasis. Maastrich, Netherlands. June 12-14, 2000.

CODEN: HMTSB7. ISSN: 0301-0147. Language: English.

L19 ANSWER 10 OF 11 CAPLUS COPYRIGHT 2009 ACS on STN
1993:119883 Document No. 118:119883 Original Reference No. 118:20705a, 20708a

Method of performing tissue **plasminogen** activator (tPA) assay.

Ranby, Mats G.; Wiman, Tor Bjoern (Biopool International, Inc., USA).

U.S. US 5175087 A 19921229, 11 pp. Cont.-in-part of U.S. Ser. No.

355,948. (English). CODEN: USXXAM. APPLICATION: US 1989-392684

19890811. PRIORITY: US 1987-70068 19870706; US 1989-355948 19890523.

AB Determination of tPA in blood with a chromogenic substrate is improved by collecting the blood in a container containing sufficient acidic buffer to lower the blood pH immediately to 5.0-6.5, and optionally addnl. a Pluronic-type surfactant to prevent excessive hemolysis. This procedure stabilizes other serine proteases, serine protease inhibitors, coagulation factors, soluble fibrin, etc. which may also be determined in the blood sample. Thus, 0.5 mL 0.5M Na citrate buffer (pH 4.3) in a siliconized blood collection tube was lyophilized. When 4.5 mL blood was drawn into the tube, the solid buffer rapidly dissolved and reduced the blood pH to .apprx.5.7.

L19 ANSWER 11 OF 11 BIOSIS COPYRIGHT (c) 2009 The Thomson Corporation on STN

1989:455481 Document No.: PREV198937088125; BR37:88125. DETERMINATION OF **PLASMINOGEN** ACTIVATOR INHIBITOR-1 PAI-1 IN PLASMA USING TWO DIFFERENT ANTICOAGULANTS AND METHODS. RYDZEWSKI A [Reprint author]; TAKADA Y; TAKADA A. DEP PHYSIOLOGY, HAMAMATSU UNIV SCH MED, HAMAMATSU 431-31, JAPAN. Thrombosis Research, (1989) Vol. 55, No. 2, pp. 285-290. CODEN: THBRAA. ISSN: 0049-3848. Language: ENGLISH.

=> s 18 and lysine

L20 940 L8 AND LYSINE

=> s 120 and plasminogen

L21 940 L20 AND PLASMINOGEN

=> s 121 and alanine

L22 45 L21 AND ALANINE

=> s 122 and imidazole

L23 1 L22 AND IMIDAZOLE

=> d 123 cbib abs

L23 ANSWER 1 OF 1 EMBASE COPYRIGHT (c) 2009 Elsevier B.V. All rights reserved on STN

2003347955 EMBASE Hydrolysis of **fibrinogen** and **plasminogen**

by immobilized earthworm fibrinolytic enzyme II from Eisenia fetida.

Zhao, Jing; Li, Li; Wu, Cen; He, Rong-Qiao (correspondence). Lab. of Vis. Information Processing, Institute of Biophysics, Ctr. for Brain/Cognitive Sciences, Beijing, China. herq@sun5.ibp.ac.cn. Zhao, Jing. School of Life Sciences, Liaoning Normal University, Dalian 116029, China.

International Journal of Biological Macromolecules Vol. 32, No. 3-5, pp. 165-171 Sep 2003.

Refs: 45.

ISSN: 0141-8130. CODEN: IJBMDR.

Pub. Country: Netherlands. Language: English. Summary Language: English.

Entered STN: 20030911. Last Updated on STN: 20030911

AB Earthworm fibrinolytic enzyme II (EFE-II) from Eisenia fetida has a broad hydrolytic specificity for peptide bonds. Our experiments show that EFE-II can hydrolyze the specific chromogenic substrates of thrombin (Chromozym TH), trypsin (Chromozym TRY) and elastase (Chromozym ELA). The Michaelis-Menten constant (K_m) for Chromozym ELA (.apprx.245 μ M) is

much higher than those for the thrombin (.apprx.90 μ M) and trypsin (.apprx.60 μ M) substrates. On the other hand, EFE-II is inhibited most strongly by soybean trypsin inhibitor (SBTI), and weakly inhibited by elastinal, suggesting that EFE-II has a trypsin-like activity.

Degradation of **plasminogen** (PLg) and **fibrinogen** by EFE-II was investigated after EFE-II had been immobilized onto 1,1'-carbonyl-diimidazole (CDI)-activated Sepharose CL-6B. The immobilized EFE-II has 55-60% activity of the native enzyme with a higher thermal and pH resistance. EFE-II cleaves PLg at four hydrolytic sites: Lys (77)-Arg(78), Arg(342)-Met(343), Ala (444)-Ala(445) and Arg(557)-Ile(558). The site Arg(557)-Ile(558) is also recognized and cleaved by tissue **plasminogen** activator (t-PA) and urokinase (UK), producing active plasmin. Cleaving Ala(444)-Ala(445) released mini-plasmin with secondary activity to hydrolyze fibrin. Immobilized EFE-II degrades not only the A α chain of **fibrinogen** in the C-terminal region (like human neutrophil elastase, HNE), but also in the N-terminal region at the Val (21)-Glu(22) site. .COPYRGT. 2003 Elsevier B.V. All rights reserved.

=> s 121 and pH 7.5
L24 4 L21 AND PH 7.5

=> dup remove 124
PROCESSING COMPLETED FOR L24
L25 4 DUP REMOVE L24 (0 DUPLICATES REMOVED)

=> d 124 1-4 cbib abs

L24 ANSWER 1 OF 4 CAPLUS COPYRIGHT 2009 ACS on STN
1991:171295 Document No. 114:171295 Original Reference No. 114:28805a, 28808a
Methods for recovery of intact tissue **plasminogen** activator using metal chelate adsorbent and chaotropic agent. Rice, Craig; Morser, Michael J.; Glaser, Charles; Donner, Peter A. (Codon Corp., USA). U.S. US 4960702 A 19901002, 20 pp. Cont.-in-part of U.S. Ser. No. 76,682, abandoned. (English). CODEN: USXXAM. APPLICATION: US 1988-167061 19880311. PRIORITY: US 1985-773334 19850906; WO 1986-US1831 19860905; US 1986-76682 19860906.

AB Intact tissue **plasminogen** activator (t-PA) is recovered from a liquid medium containing it by (a) contacting the liquid medium with a metal chelate adsorbent; (b) eluting degraded t-PA; and (c) eluting the intact t-PA with, e.g. a chaotropic agent. The purified t-PA is useful for treating a host in need of thrombolytic therapy. Human t-PA produced by recombinant human melanoma cells was purified from conditioned clarified media using a chelating Sepharose column complexed with Zn. Degraded t-PA was eluted with Tris-HCl (**pH 7.5**) buffer containing 0.1 M imidazole, etc. The intact t-PA was recovered with buffer containing 50 mM Na EDTA and then further treated with **lysine**-Sepharose to remove addnl. contaminants. The product contained .apprx.95% single-chain t-PA. Intact t-PA activity was greatly stimulated by **fibrinogen** fragments; the 2-chain form and degraded forms were stimulated to a lesser extent.

L24 ANSWER 2 OF 4 CAPLUS COPYRIGHT 2009 ACS on STN
1975:90061 Document No. 82:90061 Original Reference No. 82:14373a, 14376a
Fibrinogen. Asada, Toshio; Igarashi, Michiko; Matsumoto, Komin; Urita, Yuzo; Ohkubo, Yosiko (Daiichi Seiyaku Co., Ltd.). Jpn. Kokai Tokkyo Koho JP 49019011 19740220 Showa, 4 pp. (Japanese). CODEN: JKXXAF. APPLICATION: JP 1972-59033 19720615.

AB A dilute solution of crude **fibrinogen** (I) was purified by passing through a **lysine**-agarose or **lysine**-polyacrylamide column. Thus, 0.4% cattle I solution (0.005 M phosphate buffer containing 0.85%

NaCl, **pH 7.5**) was passed through a **lysine**-agarose column (1 ml), and EtOH added to the effluent up to 10% to precipitate I free from **plasminogen**. I was stored after freeze-drying in the above-mentioned buffer containing 0.2% Na citrate. Human I was similarly prepared. The **lysine**-agarose was prepared treating 10 ml Sepharose 4B with 1 g BrCN and then with 2 g L-**lysine** at an alkaline pH.

L24 ANSWER 3 OF 4 CAPLUS COPYRIGHT 2009 ACS on STN
1974:57111 Document No. 80:57111 Original Reference No. 80:9273a, 9276a
Separation and measurement of **plasminogen** activators from tissues and body fluid by affinity chromatography. Shiba, Tadaaki (Med. Sch., Toho Univ., Tokyo, Japan). Toho Igakkai Zasshi, 20(1/2), 88-93 (Japanese) 1973. CODEN: TOIZAG. ISSN: 0040-8670.

AB **Plasminogen** activators were adsorbed on a **lysine**-Sepharose column equilibrated with 0.005M phosphate buffer (**pH 7.5**) and were eluted with 0.005M phosphate buffer (**pH 7.5**) containing M NaCl. **Plasminogen** was retained on the column under these conditions. Both fibrin plates containing **plasminogen** and fibrin plates containing **plasminogen**-free **fibrinogen** prepared by affinity chromatog. on **lysine**-Sepharose columns were useful for the determination of **plasminogen** activation.

L24 ANSWER 4 OF 4 CAPLUS COPYRIGHT 2009 ACS on STN
1963:22037 Document No. 58:22037 Original Reference No. 58:3685a-b
Estimation of fibrinolytic activity in human plasma by heparin fractionation. Green, J.; Thompson, W. B. (Paddington Gen. Hosp., London). Biochemical Journal, 84, 74P-75P (Unavailable) 1962. CODEN: BIJOAK. ISSN: 0264-6021.

AB Plasma is diluted with 2 vols. of H₂O containing 12.5 units of heparin/ml. and the pH is adjusted to 6.8; the heparin complex, which contains **fibrinogen** and **plasminogen**, is centrifuged, washed, and redissolved in barbiturate-NaCl buffer, **pH 7.5**, ionic strength 0.15. It can be used for visual determination of lytic activity after clotting by thrombin, determination of proteolytic activity by measuring the extinction at 280 m μ after precipitation, and esterolytic activity towards arginine and **lysine** esters.

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|--|------------------|---------------|
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